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Neuronal Control of Heartbeat in the Medicinal Leech

II. Intersegmental Coordination of Heart Motor Neuron Activity by Heart Interneurons

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Summary. The coordinated activity cycles of the heart excitor motor neurons, or HE cells, of the leech ventral nerve cord responsible for the heart tube constriction rhythm are controlled by a set of heart interneurons, or HN cells. Ganglia 3, 4, 6 and 7 of the ventral cord each contain a pair of such HN cells, which send axons into the posterior connective. On their rearward course, the HN cell axons transit through the neuropil of many posterior ganglia where they inhibit the ipsilateral HE cells. The HN cells maintain an activity rhythm consisting of an active phase, during which they are depolarized and produce a burst of impulses, and an inactive phase during which they are repolarized while receiving a burst of inhibitory synaptic potentials.

The HN cells form part of the neural oscillator that is the source of the heartbeat rhythm, since the phase of the rhythm can be shifted by evoking impulses in an HN cell during the inactive phase of its activity cycle. The HN cell activity rhythms are coordinated intra- and intersegmentally in such a manner that on the peristaltic side there is a progressive rear-to-front progression in the phase of the HN cell activity cycles and that on the non-peristaltic side the activity cycles occur in phase.

A quantitative theoretical analysis shows that the pattern of HN-HE cell synaptic links and the phase relations of the HN cell activity cycles can account for the observed phase relations of the HE activity cycles and hence ultimately also for the heart tube constriction dynamics on both peristaltic and non-peristaltic body sides.

Introduction

It was shown in the first paper of this series (Thompson and Stent, 1976a) that the heartbeat of the medicinal leech, *Hirudo medicinalis*, is bilaterally asymmetric. On one side, the constriction-dilation cycle of the segmental heart tube

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sections is coordinated in a frontward peristaltic mode, whereas on the other side the heart tube sections constrict nearly in concert in a non-peristaltic mode. This bilateral asymmetry of the constriction rhythm is reversible in that sometimes the right and sometimes the left heart tube beats in the peristaltic constriction mode. The constriction-dilation cycles of the segmental heart tube sections were found to be controlled by pairs of rhythmically active motor neurons in the segmental ganglia, the heart excitors, or HE cells. 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, or ISP's. In accord with the demonstrated role of the HE cells as excitatory motor neurons to the segmental heart tube muscles, the intra- and intersegmental coordination of the relative phases of the HE cell activity cycles matches that of the heart tube constriction pattern on both the peristaltic and the non-peristaltic sides.

Detailed examination of the ISP bursts of homolateral HE cells in adjacent ganglia showed that most or all of the individual ISP's recorded from the anterior HE cell precede with one of two fixed lead times a matching ISP in the posterior HE cell. Thus it could be inferred that HE cells receive their periodic inhibitory input from two or more cells whose axons course in the interganglionic connectives and whose impulses travel rearward with different impulse conduction velocities.

The work to be reported in this second paper concerns the identification of the source of the periodic inhibitory input to the HE cells. As was found here, the interganglionic axons belong to rhythmically active pairs of heart interneurons in the ganglia in the front part of the leech ventral cord. On the peristaltic body side, the activity cycles of these interneurons are locked into a frontward phase progression, whereas on the non-peristaltic body side the activity cycles of all interneurons except the frontmost occur in nearly the same phase. Thus, the phase relation of the activity cycles of the interneurons and the inhibitory synaptic links between the interneurons and the heart excitors explain how the HE cell rhythm is produced and coordinated along the ventral cord, and how the heart tube constriction pattern is generated by the leech central nervous system.

Materials and Methods

All experiments were carried out with isolated preparations of the ventral nerve cord of *Hirudo medicinalis*, varying in length from a single segmental ganglion to a chain of 19 ganglia, as described in the first paper of this series (Thompson and Stent, 1976a). The experimental methods of maintaining the preparations, of securing intracellular electrophysiological records and of staining individual nerve cells by intracellular horseradish peroxidase injection were also those previously described.

Results

Identification of Heart Interneurons

A search for the interganglionic source of the ISP bursts in the heart excitors led to the identification in some ganglia of a bilateral cell pair whose impulse

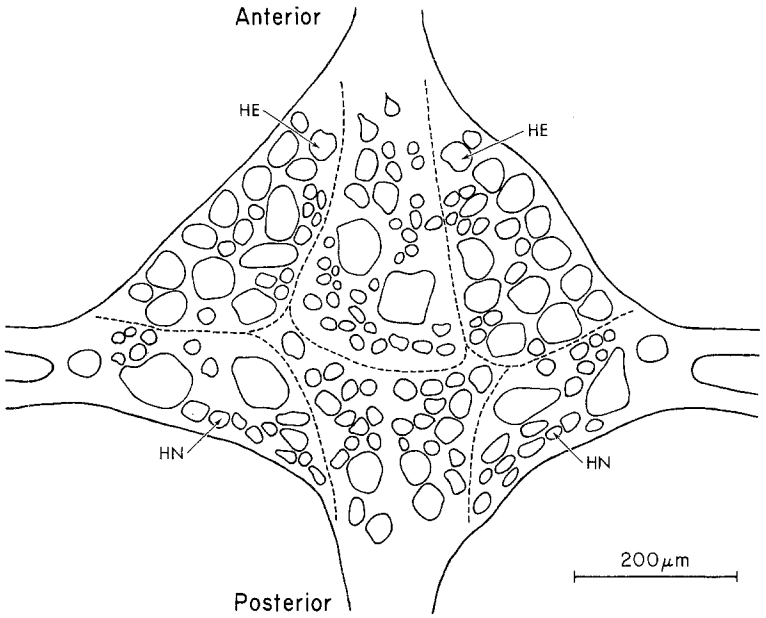

Fig. 1. Ventral aspect of a segmental ganglion in the anterior part of the leech ventral cord showing the location of the cell bodies of the heart excitor motor neuron, or HE cell, and of the heart interneuron, or HN cell. The HE cells are found in this position in ganglia 3–19. The HN cells are found in approximately this position in ganglia 2–7. The position of the HN cell body relative to neighboring cell bodies varies slightly from ganglion to ganglion and, as shown here, the HN cell pair is not necessarily distributed with perfect symmetry across the long body axis

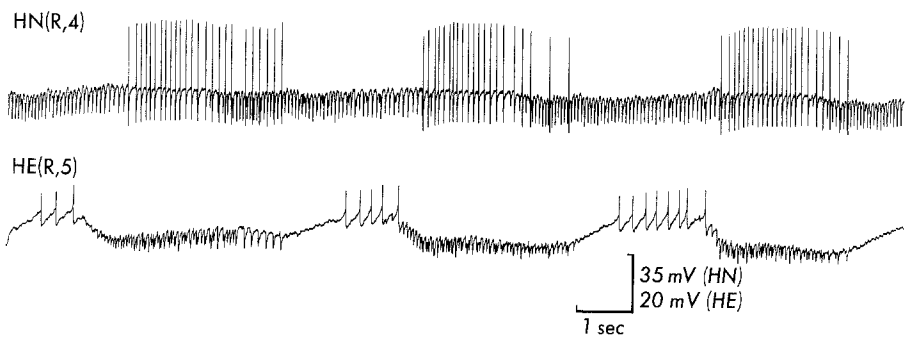

Fig. 2. Intracellularly recorded activity rhythms of a heart interneuron and of an HE cell to which the HN cell provides inhibitory synaptic input

bursts give rise to ISP bursts in HE cells. These rhythmically active HE cell inhibitors were designated as heart interneurons, or HN cells. (That the HN cells are interneurons whose processes project only within the central nervous system will be justified later on morphological grounds). Thus far, HN cell pairs have been identified in ganglia 3, 4, 6 and 7, whereas intensive surveys

of ganglia 8, 9 and 10 failed to reveal their presence. Ganglia 2 and 5 also contain heart interneuron pairs, but their function, which will be described in the following paper (Thompson and Stent, 1976b), is not to provide ISP bursts to the HE cells. The HN cell body is located at the lateral edge of the posterior lateral cell packet on the ventral aspect of the ganglion, as shown diagrammatically in Figure 1.

A record of the periodic activity pattern of a heart interneuron is presented in Figure 2. Both the interneuron cell HN(R,4), and the motor neuron HE(R,5), alternate rhythmically between an active and an inactive phase, with a period which is equal to the heartbeat period. During the active phase of its activity cycle the HN cell generates an impulse burst and during the inactive phase the HN cell receives a burst of ISP's. As can be seen, the active phase of cell HN(R,4) coincides roughly with the inactive phase of cell HE(R,5). This antiphasic relation is in accord with the findings to be presented that cell HN(4) is a source of ISP bursts in the ipsilateral cell HE(5).

The records of Figure 3 show first, that each action potential in cell HN(R,3) was followed by an ISP in the ipsilateral cell HE(R,3) after a delay of 4 ms. Furthermore, transient suppression of the presynaptic action potentials by passage of a pulse of hyperpolarizing current into cell HN(R,3) caused a concomitant reduction of the ISP frequency in cell HE(R,3). Similar data not presented here have shown that HN(3) cell action potentials were followed by ISP's also in the ipsilateral cell HE(4) and cell HE(7) with constant delays. Hence, cell HN(3) appears to send an axon into the posterior connective at least as far as ganglion 7 and in its rearward course to make inhibitory synapses onto the posterior, ipsilateral HE cells. Second, Figure 3 shows that each action potential occurring in cell HN(4) is followed by an ISP in the ipsilateral cell HE(5) after a delay of 40 ms and in the ipsilateral cell HE(13) after a delay of 256 ms. In order to determine whether these connections of HN cells to HE cells are monosynaptic or polysynaptic, via one or more interposed neurons, 20 mM Ca^{++} plus 20 mM Mg^{++} was added to the normal bathing fluid of the preparation. Under these ionic conditions polysynaptic connections in the leech CNS are blocked while monosynaptic potentials persist (Nicholls and Purves, 1970). Figure 3 shows that under these ionic conditions each HN(4) cell action potential is still followed after a constant delay by an HE(13) cell ISP, suggesting that the HN cell–HE cell connection is monosynaptic. (It is to be noted that, in the presence of the high Ca^{++} and Mg^{++} concentrations, the HN cell impulse to HE cell ISP delay is increased by 40%, very likely due to a decrease in the impulse conduction velocity of the HN cell axon caused by the high concentration of divalent cations).

Other records not presented here show that individual action potentials in cell HN(4) are followed by ISP's in the ipsilateral cells HE(7), HE(8), and HE(10) but not in cells HE(3) or HE(4). Hence it may be concluded that cell HE(4) sends its axon into the posterior connective at least as far rearward as ganglion 13 and that, as does cell HN(3), it makes inhibitory synapses with the ipsilateral HE cells located in posterior ganglia. In contrast to cell HN(3), however, cell HN(4) does not make inhibitory synapses with the HE cell of its own ganglion.

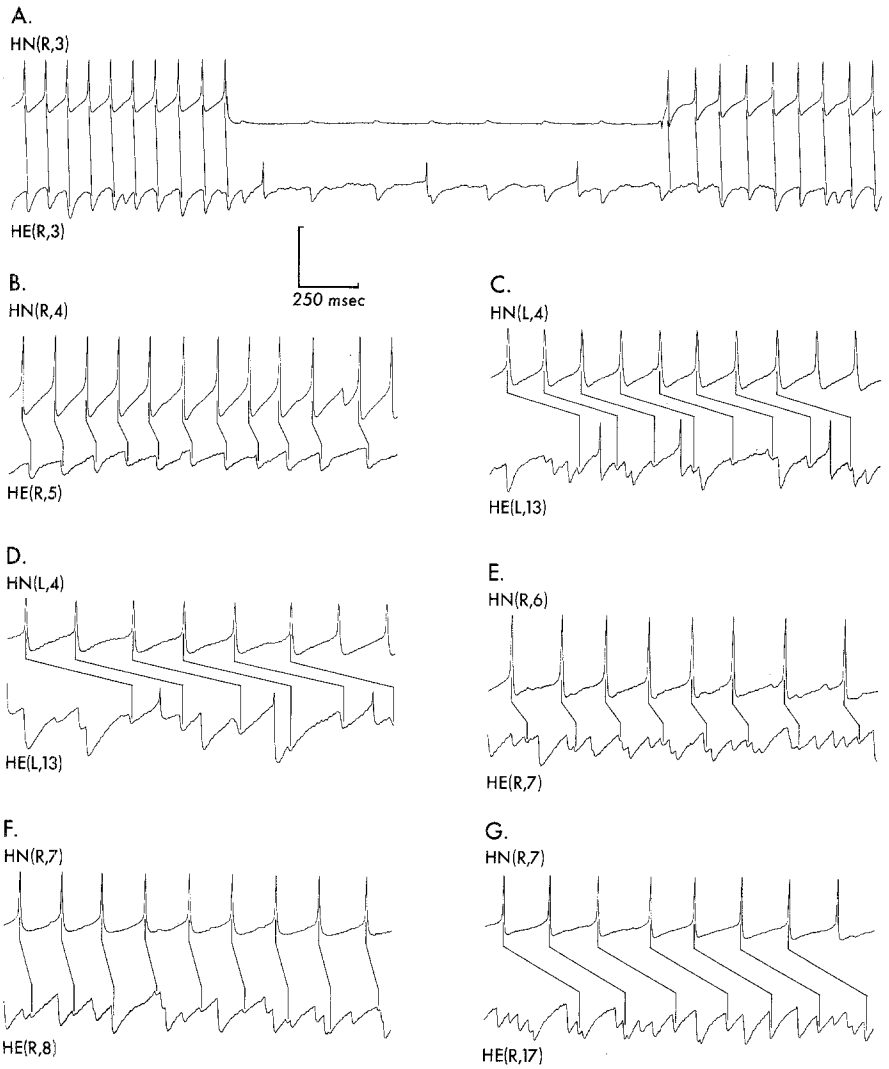

Fig. 3. Identification of inhibitory synapses made by HN cells onto HE cells. Each action potential in the HN cell is matched by a connecting line with an ISP following it with constant delay in the HE cell. During the middle part of the record shown in Panel A hyperpolarizing current was passed into the HN cell. While the record of Panel D was being taken, the preparation was bathed in saline containing 20 mM Mg^{++} and 20 mM Ca^{++}. Vertical calibration mark – panels A and B, HN cell record: 38 mV, HE cell record: 12 mV; panels C and D, HN cell record: 28 mV, HE cell record: 12 mV; panel E, HN cell record: 36 mV, HE cell record: 21 mV; panel F, HN cell record: 21 mV; HE cell record: 16 mV; panel G, HN cell record: 38 mV; HE cell record: 12 mV

Figure 3 further shows that cells HN(6) and HN(7) resemble cells HN(3) and HN(4) in that each of their action potentials is followed with a fixed delay by an ISP in a posterior, ipsilateral HE cell. The rearward range of the cell HN(7) axon extends at least as far as ganglion 17. Data from other

Table 1. Survey of synaptic links from HN cells to HE cells

HN cell tested	HE cell tested	Type of connection found	Delay between HN cell impulse and HE cell synaptic potential (ms)
R,3	R,3	Inhibitory (ISP)	4
L,3	L,3	Inhibitory (ISP)	4
R,3	R,4	Inhibitory (ISP)	40
R,3	R,7	Inhibitory (ISP)	112
L,3	R,3	No connection	
L,3	L,17	No connection	
R,4	R,5	Inhibitory (ISP)	24
R,4	R,5	Inhibitory (ISP)	40
L,4	L,7	Inhibitory (ISP)	112
R,4	R,8	Inhibitory (ISP)	104
R,4	R,8	Inhibitory (ISP)	120
L,4	L,10	Inhibitory (ISP)	160
L,4	L,13	Inhibitory (ISP)	304
L,4	L,13	Inhibitory (ISP)	256[a]
R,4	R,18	Excitatory (ESP)	560[b]
R,4	R,3	No connection	
R,4	R,4	No connection	
L,4	L,4	No connection	
L,4	R,17	No connection	
R,4	L,17	No connection	
R,6	R,6	No connection	
R,6	R,7	Inhibitory (ISP)	52
R,7	R,8	Inhibitory (ISP)	24
R,7	R,8	Inhibitory (ISP)	40
R,7	R,8	Inhibitory (ISP)	32
R,7	R,17	Inhibitory (ISP)	232
R,7	R,17	Inhibitory (ISP)	296
R,7	R,18	Inhibitory (ISP)	336
R,7	R,3	No connection	
R,7	L,7	No connection	
L,7	L,7	No connection	
L,7	R,7	No connection	
R,7	L,18	No connection	

Each entry represents the result of simultaneous intracellular recordings taken from an HN cell and an HE cell. A synaptic link was inferred to exist if synaptic potentials in the HE cell followed with constant delay action potentials in the HN cell. No reverse connections from HE to HN cells were found

[a] ISP's persisted in saline containing 20 mM Mg^{++} and 20 mM Ca^{++} with a delay of 384 ms

[b] ESP's persisted in saline containing 20 mM Mg^{++} and no Ca^{++} with a delay of 720 ms

recordings not presented here show that the rearward range of the cell HN(6) axon extends at least as far as ganglion 10, and that cells HN(6) and HN(7) do not synapse onto the HE cells of their own ganglia 6 and 7, respectively.

A survey carried out to ascertain the rearward range of the inhibitory synaptic contacts made by the HN cell axon on posterior HE cells showed that cell

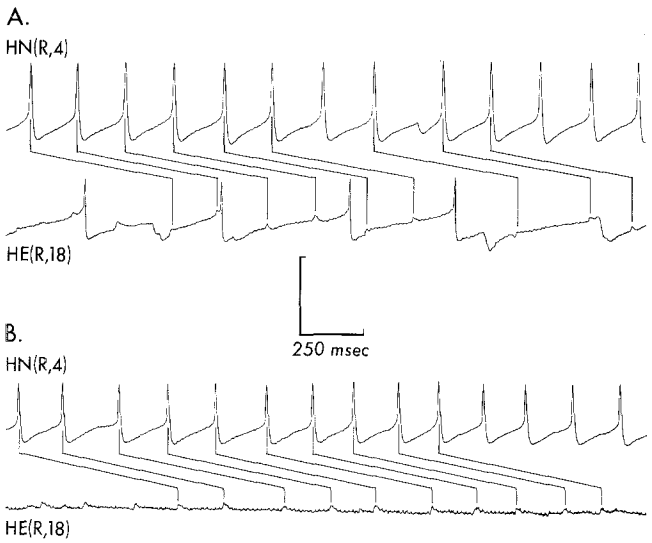

Fig. 4. Identification of an excitatory synaptic connection between an HN cell and a caudal HE cell. Panel A: Action potentials in cell HN(R,4) are matched by connecting lines with ESP's in cell HE(R,18). Panel B: Same preparation as in panel A, except that the preparation is bathed in a Ca^{++}-free saline containing 20 mM Mg^{++}. Vertical calibration mark – HN cell record: 50 mV; HE cell record: 20 mV

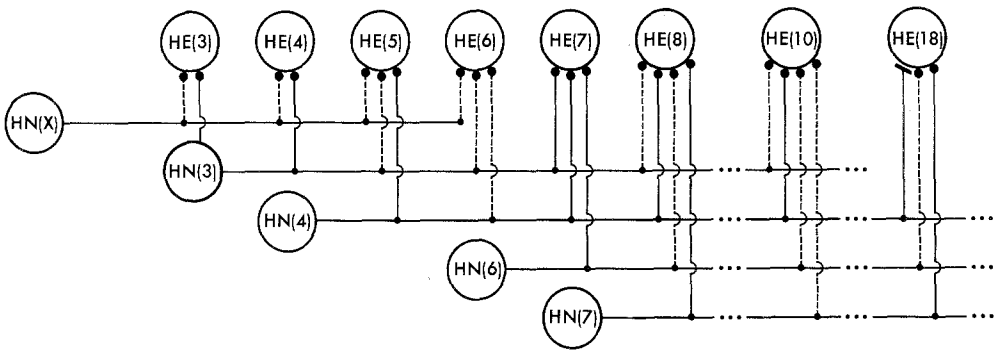

Fig. 5. Hemilateral circuit diagram showing the connections between HN and HE cells. The labeled circles represent the particular nerve cell bodies and the lines the cell processes. Filled circles represent inhibitory synapses and T-joints excitatory (electrical) junctions. Solid lines indicate connections established by records taken simultaneously from the pre- and postsynaptic neurons. Dashed lines indicate connections which are known to exist by other criteria. The circuit is duplicated on the other body side

HN(3) and cell HN(4) do not provide ISP input to either the ipsi- or the contralateral cell HE(17) and cell HE(18) at the caudal end of the ventral cord. However, this survey also brought the rather unexpected result presented in Figure 4, namely that each action potential in cell HN(R,4) is followed in cell HE(R,18) by an excitatory synaptic potential (ESP), rather than by an ISP, after a delay of 560 ms. In order to test whether these ESP's arise

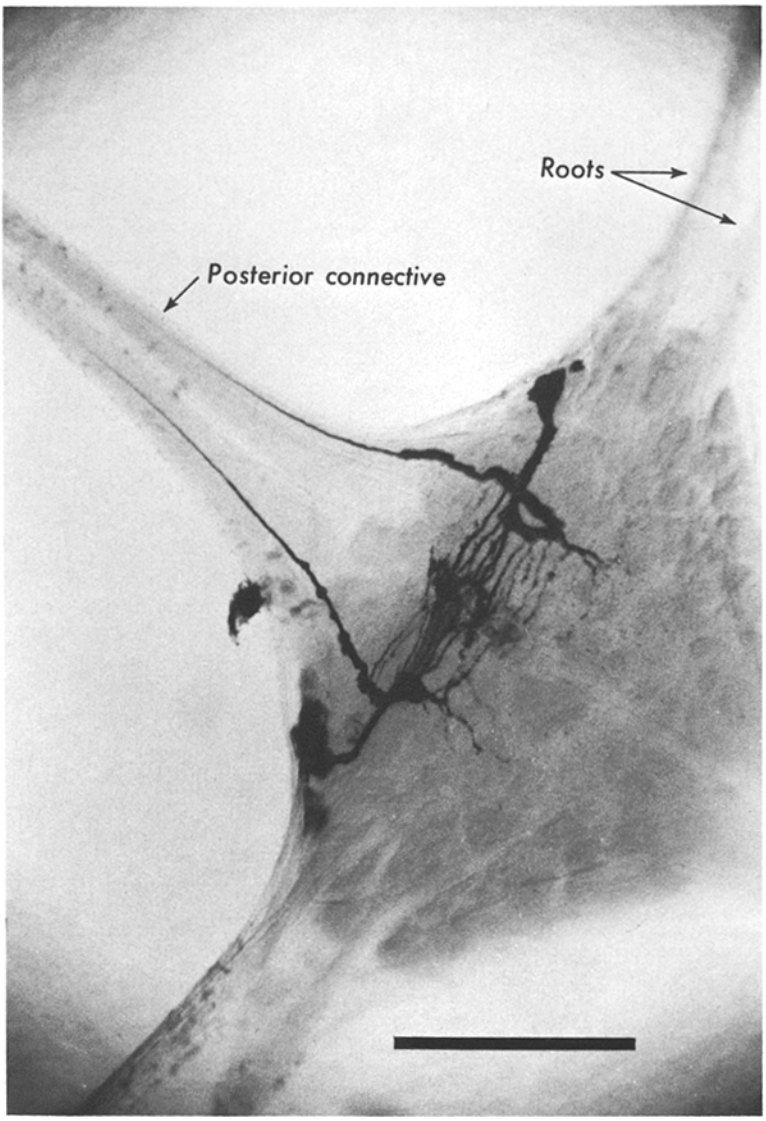

Fig. 6. Anatomy of the heart interneuron. Photograph taken from the ventral aspect of ganglion 4, in which the HN cell pair was stained by intracellular injection of horseradish peroxidase. Distance marker about 200 μm

from electrical or chemical synaptic contacts, the preparation was bathed in Ca^{++}-free saline containing 20 mM Mg^{++}, which blocks chemical but not electrical synaptic transmission in the leech ganglion (Nicholls and Purves, 1970). As can be seen in Figure 4 replacement of Ca^{++} by Mg^{++} in the bathing fluid abolished the ISP's in cell HE(R,18) but not the ESP's which follow each action potential in cell HN(R,4). It can be concluded, therefore, that

the axon of cell HN(4) makes an electrical junction onto the ipsilateral cell HE(18), and thus that the character of the synaptic contact between cell HN(4) and the ipsilateral HE cells changes from inhibitory to excitatory at a ganglion posterior to ganglion 13. However, no similar electrical junctional contact between the axon of cell HN(3) and the ipsilateral cell HE(17) or cell HE(18) was found. The axon of cell HN(3) either does not reach as far posteriorly as ganglion 17, or, if it does, it makes no discernible connections with the caudal HE cells.

The results of a general survey of the connections between HN cells and HE cells are summarized in Table 1 and in the circuit diagram of Figure 5. Since not all of the more than 250 possible pairwise combinations of right and left HN cells and HE cells have as yet been examined, the assumption has been made in the construction of this circuit diagram that the nature of these contacts is bilaterally symmetric and varies in a systematic manner from front to rear. That is to say, it has been assumed that if the nature of the synaptic contact between an HN cell axon and the HE cell is the same in both an anterior and a posterior ganglion, then it is also of that same nature in all ganglia lying between the two ganglia for which the test has been made. As can be seen in this circuit diagram, the HN cell axons make synaptic contact only with ipsilateral HE cells in ganglia posterior to the ganglion in which the HN cell body is located [except for cell HN(3) which contacts also the ipsilateral cell HE(3)].

The photomicrograph of Figure 6 presents the result of staining the HN cell pair of ganglion 4 by intracellular injection of horseradish peroxidase. Similar pictures have been obtained for the HN cell pair of ganglion 3 and ganglion 7. The single process that emerges from the HN cell body can be seen to form a loop in the ipsilateral neuropile and to exit from the ganglion by way of the ipsilateral posterior connective. In agreement with the electrophysiological findings that the HN cells do not make synaptic contacts in ganglia anterior to the location of their cell bodies, no stained HN cells have been seen to project axons into the anterior connective. Furthermore, since the HN cells send no processes into the segmental nerve roots, their designation as "interneurons" is justified on anatomical grounds. Within the neuropile the HN cell gives off a profuse arborization of fine processes, which are directed towards the midline of the ganglion. At the midline, the fine processes of right and left HN cells appear to mesh. The camera lucida drawings of Figure 7 of the processes of another, similarly stained HN cell show that its axon transits directly through the neuropile of the next posterior ganglion. It is to be noted, however, that here the extensive arborization and meshing of fine processes at the ganglionic midline seen in the ganglion of origin of the HN cell axon is not visible in neuropile of the next posterior ganglion, where only a few small processes appear to emerge from the HN cell axon. This suggests that the synaptic connections between the transiting heart interneuron and the heart excitors of posterior ganglia derive from contacts of the fine HE cell processes (cf. Fig. 6 of Thompson and Stent, 1976a) with the main, unbranched HN cell axon.

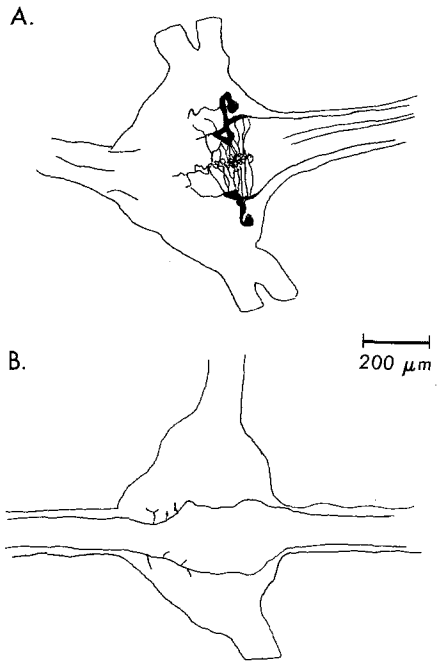

Fig. 7. Anatomy of heart interneuron processes. Camera lucida drawings of two adjacent ganglia containing the processes of the HN cell pair of the anterior ganglion stained by intracellular injection of horseradish peroxidase. Panel A: The cell bodies and processes of the HN(7) cell pair in ganglion 7. Panel B: The processes of the HN(7) cell pair in ganglion 8

Activity Patterns of the Heart Interneurons

The coordinated activity of the HN cells was ascertained indirectly, by analysis of the ISP burst pattern observed in the HE cell ensemble, and directly, by comparing records obtained from the HN cells themselves. Whereas the interpretation of the HN cell activity records obtained by the direct procedure is more straightforward, the results obtained indirectly by the interpretation of HE cell records are free of possible artifacts produced by electrode penetration of HN cells. An example of the indirect procedure is presented in Figure 8, which shows a single ISP burst recorded concurrently from cell HE(L,7) and cell HE(L,9). The cells were evidently coordinated in the peristaltic mode, since the activity cycle of the posterior HE cell leads in phase that of the anterior HE cell. Analysis of various portions of the record of the ISP burst shows that every ISP in the anterior HE cell can be matched with a later ISP in the posterior HE cell, as in Figure 11 of the preceding paper (Thompson and Stent, 1976a). However, there occur some ISP's in the posterior HE cell which have no correspondent in the anterior HE cell. As shown in Figure 5, the HN cells of ganglion 4 and ganglion 7 form inhibitory synapses onto the ipsilateral HE cells of more posterior ganglia, but do not synapse with the HE cells of their own ganglia. Hence, ISP's attributable to impulses in the axons of cell HN(3), cell HN(4) and cell HN(6) would occur both in cells HE(7) and

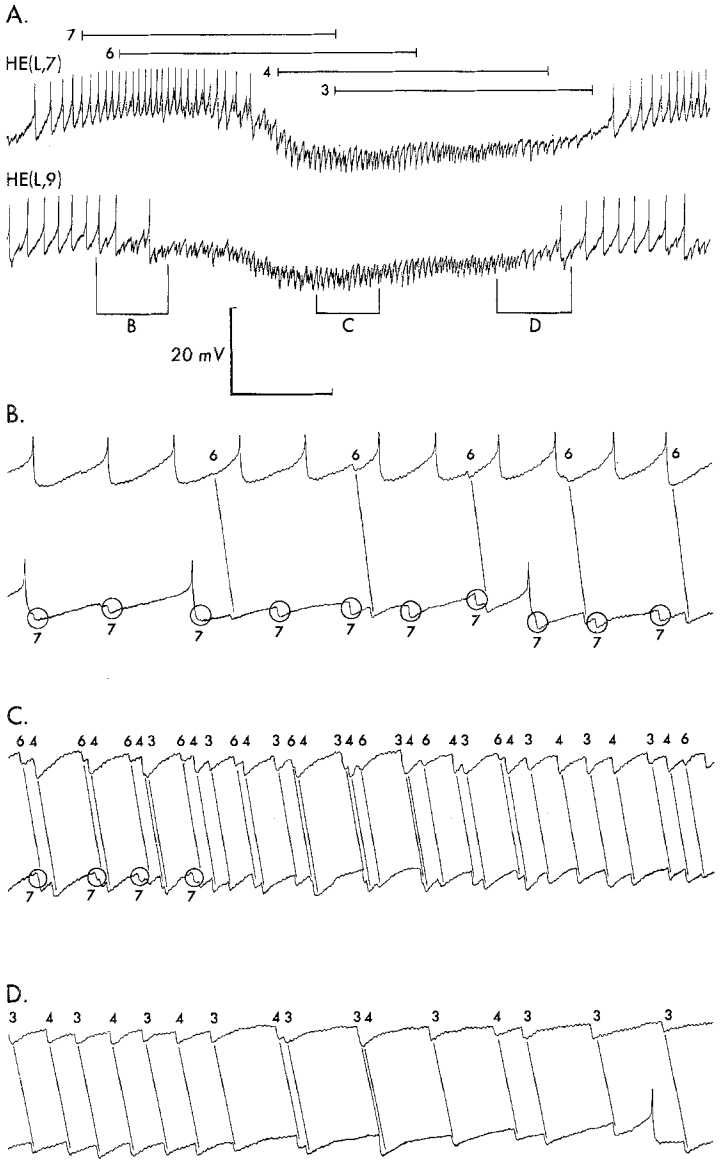

Fig. 8. The indirect method for ascertaining the coordination of the activity rhythms of the HN cell ensemble by analysis of the HE cell ISP burst pattern. Panel A: a single ISP burst in cells HE(L,7) and HE(L,9). The bars at the top of panel A identify the active phases of the cycles of the HN cells, as determined by the occurrence of individual ISP's produced by their impulses. The brackets at the bottom of panel A identify the portions of the record which are expanded further in other panels of this figure. Panel B: expansion of the initial portion of the ISP burst record of panel A. Numbers identify the HN cell responsible for each ISP. ISP's occurring in HE(L,9) with no correspondent in HE(L,7) are due to HN(L,7) impulses and are circled in this panel and in panel C. Panel C: expansion of a central portion of the ISP burst record of panel A. Panel D: expansion of the latter portion of the ISP burst record of panel A. Horizontal calibration mark indicates 4 s in panel A and 400 ms in panels B, C, and D

HE(9); impulses in the cell HN(7) axon would produce ISP's in cell HE(9) but not in cell HN(7). Thus, unmatched ISP's which occur in cell HE(9) but not in HE(7) identify impulses of cell HN(7). It may be inferred from the times of occurrence of such unmatched ISP's in the traces of Figure 8 that HN(7) cell impulses begin early in the ISP burst of cell HE(9), before any activity has begun in any of the other HN cells which make inhibitory synapses onto cell HE(9) and cease approximately midway through the ISP burst. It appears, therefore, that the phase of the activity cycle of cell HN(7) leads the phases of the cycles of cells HN(3), HN(4), and HN(6).

The timing of HN(6) cell impulses, which, according to the circuit diagram of Figure 5, produce ISP's in cell HE(7) but not in cell HE(6), and of the HN(4) cell impulses, which produce ISP's in cell HE(5) but not in cell HE(4), was ascertained by applying the same procedure of identifying unmatched ISP's in concurrent records obtained from other homolateral HE cell pairs (not presented here). In this way, the relative phasing of the impulse bursts in cells HN(3), HN(4) and HN(6) could be established. This analysis showed that the phase of the activity cycle of cell HN(6) leads that of the ipsilateral cell HN(4), which in turn leads that of the ipsilateral cell HN(3). The relative timing of the active phases of the heart interneuron activity cycles in the peristaltic coordination mode, as determined in this way, is presented graphically in Figure 9. Evidently, there is a regular frontward phase progression of the heart interneuron activity cycles, similar to the frontward progression in heart tube constriction cycles in the anterior segments of the peristaltic side.

As was shown in the preceding paper (Thompson and Stent, 1976a) the delay observed between matching ISP's occurring in pairs of homolateral HE cells may fall into several classes. This finding was interpreted to mean that the impulses responsible for these ISP's travel in a variety of interganglionic axons with different conduction velocities. In the light of the circuit diagram of Figure 5 it would now follow that the axons of different HN cells have different impulse conduction velocities, and that, therefore, the delay observed between two matching ISP's is an indicator of the particular HN cell whose impulse had produced them. This differential delay criterion now allows the identification of component ISP's due to individual HN cells within the mixed HE cell ISP bursts presented in Figure 8. Since, as has been inferred, the active phase of cell HN(6) precedes that of the ipsilateral HN(3) and HN(4) cells, it is possible to identify the class of ISP's due to HN(6) impulses in the ISP burst of the homolateral HE cell pair: the first matching ISP's that occur in the burst are evidently due to cell HN(6) impulses and their intersegmental delay provides the signature for later ISP's due to that cell. As soon as there appear matching ISP's in the records which are separated by a delay different from that characteristic of cell HN(6) impulses, it can be inferred that they are due to impulses of cell HN(4). Finally, the last matching ISP class to appear with a characteristic delay must be due to impulses in cell HN(3). By use of this procedure every ISP seen in the expanded records of the ISP bursts of Figure 8 could be assigned to an impulse in a particular HN cell.

Detailed analysis of matching ISP's in the series of homolateral HE cells revealed, however, that there occurs in cells HE(3), HE(4), HE(5) and HE(6)

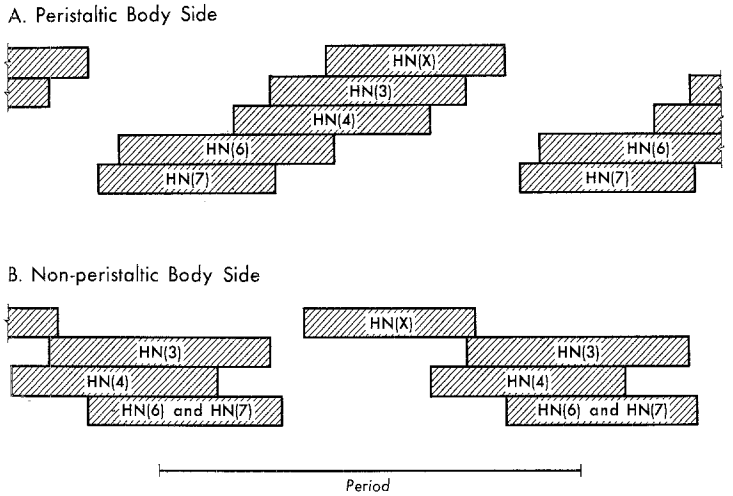

Fig. 9. Schematic summary of the relative phase relations of HN cell activity cycles on the two body sides, as determined by the indirect method of HE cell ISP burst pattern analysis illustrated in Figure 8. Each cross-hatched rectangle represents the active phase of the HN cell indicated. The data obtained from different preparations were normalized to a standard period of 15 s

an additional class of ISP's which is not attributable to impulses in either cell HN(3) or cell HN(4). These additional ISP's have a small amplitude, particularly in the HE cells posterior to ganglion 4, and occur at regular intervals in a burst pattern. Thus, there exist one or more additional, as yet unidentified HN cells which for the sake of simplifying the discussion will be considered as a single cell and referred to as cell HN(X). The phase of the activity cycle of cell HN(X) lags behind that of cell HN(3).

Once the existence of the unidentified cell HN(X) is taken into account, all visible ISP's in the HE cells of ganglia 3 through 9 can be accounted for by impulses travelling in the axons of cells HN(X), HN(3), HN(4), HN(6), and HN(7).

The phase relations of the activity cycles of the HN cells on the two body sides were ascertained by examining ISP's in records obtained from bilateral pairs of HE cells in the same ganglion, such as the records presented in Figure 8 of the preceding paper (Thompson and Stent, 1976a). For instance, the large amplitude ISP's recorded from either HE(4) cell represent synaptic input from the ipsilateral HN(3) cell. Since the phase angle between the bursts of these ISP's in right and left HE cells is about 180 °, the activity cycles of right and left HN(3) cells must be separated also by about 180 °. The small amplitude ISP's recorded from either HE(4) cell represent synaptic input from the unidentified HN(X) cell. The bursts of these small amplitude ISP's due to HN(X) impulses occur in phase in right and left HE(4) cells. However, since the individual small amplitude ISP's seen in cell HE(L,4) do not match those seen in cell HE(R,4), it follows there must exist an HN(X) cell pair, of which one member supplies ISP's to the HE cells on the left and the other to the HE cells on the right body side, with the activity cycles of both HN(X) cells apparently being in phase.

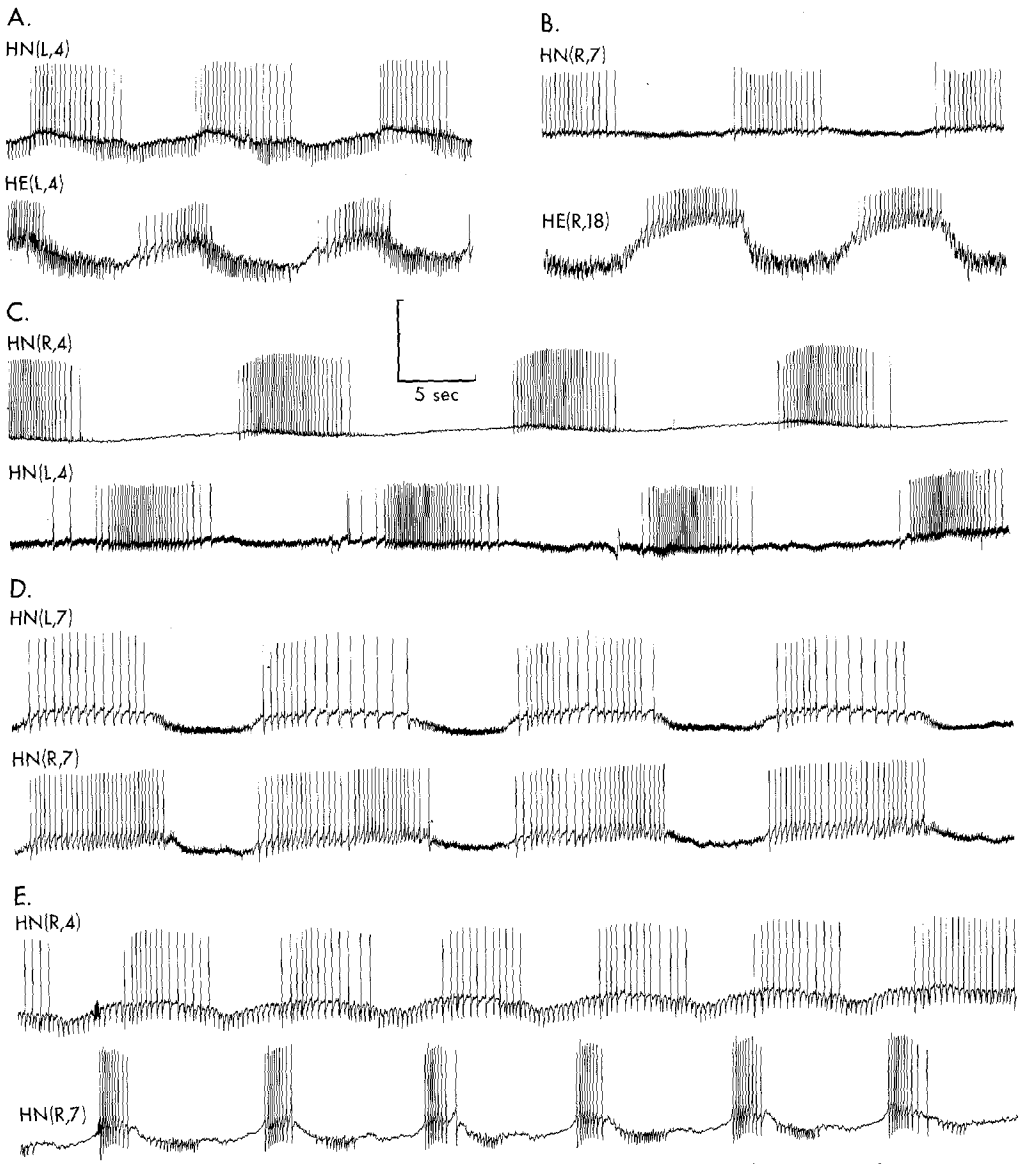

Fig. 10. The direct method for ascertaining the coordination of the activity rhythm of the HN cell ensemble by simultaneous recording from an HN cell and an HE cell, or from two HN cells. Vertical calibration mark—panels A and B, HN cell record: 29 mV; HE cell record: 32 mV; panel C: HN(R,4) cell record: 29 mV; HN(L,4) cell record: 22 mV; panel D: HN(L,7) cell record: 36 mV; HN(R,7) cell record: 25 mV; panel E: both records: 33 mV

A summary of the relative phase relations of HN cell activity cycles as determined by analyzing ISP's in HE cells in the peristaltic and non-peristaltic modes of coordination is presented in Figure 9. This summary is based on HE cell activity records obtained from four different preparations, the ISP burst data having been normalized to a standard period and averaged. Since

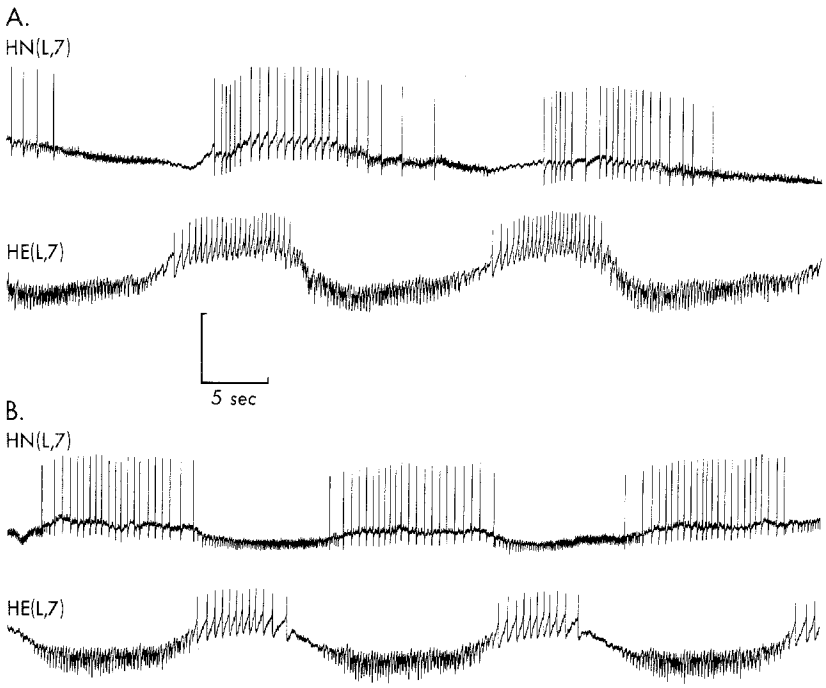

Fig. 11. Peristaltic and non-peristaltic coordination modes of the activity cycles of a heart interneuron and a heart excitor. Panel A: record taken while left side is in peristaltic coordination mode. Panel B: later record taken while left side is in non-peristaltic coordination mode. Vertical calibration mark – HN cell record: 30 mV; HE cell record: 15 mV

no recordings of the cell pairs HE(6)–HE(7) or HE(7)–HE(8) in the non-peristaltic coordination mode were available, only the combined activity cycles of cells HN(6) and HN(7) have been identified.

The back-to-front peristalsis of the heart tube on the peristaltic side is matched by a back-to-front phase progression of the HN cell activity cycles, with the cycle of the rearmost cell HN(7) leading that of the (presumably) frontmost cell HN(X) by about 180 °. A significantly different phase relation of the HN cell activity cycles obtains on the non-peristaltic side, although here too the phase angle between the cycles of rearmost and frontmost HN cells is about 180 °. But the cycles of the intermediate cells HN(3) and HN(4), whose phases *follow* those of cell HN(7) and HN(6) by about 130 ° on the peristaltic side, *lead* the phases of the rearmost HN cells by about 40 ° on the non-peristaltic side. Furthermore, it is to be noted that the activity cycles of the bilateral homologs of the frontmost and rearmost cells HN(X), HN(6) and HN(7) are nearly in phase, whereas a phase angle of about 180 ° is maintained between the cycles of right and left homologs of the intermediate cells HN(3) and HN(4).

Examples of the direct procedure for ascertaining the coordination of the HN cell activity rhythm, namely recording from HN cells themselves, are presented in Figure 10. These records generally confirm the inferences derived by means of the indirect procedure of HE cell ISP correlation summarized

in Figure 9. For instance, it can be seen that the activity cycles of cells HN(4) and HN(7) are separated by phase angles of about 180 ° from the cycles of their ipsilateral cells HE(4) and HE(18) respectively. Hence, relative to each other, cells HN(4) and HN(7) are indeed active in antiphase on the non-peristaltic side. Furthermore, it is evident that the cycles of the bilateral cell pair HN(R,4) and HN(L,4) are indeed separated by a phase angle of about 180 °, and that the cycles of the bilateral HN(L,7) and HN(R,7) cell pair indeed do occur in the same phase. Finally, the activity cycle of HN(R,7) evidently leads that of its homolateral homolog HN(R,4) by a phase angle of about 120 °, or in just the relation which was previously inferred to obtain on the peristaltic side.

It follows from the phase relations diagrammed in Figure 9 that the alternation between the peristaltic and the non-peristaltic heartbeat mode derives from changes in the coordination of the activity cycles of the set of ipsilateral HN cells. Such changes in coordination happened to occur in a few cases while recordings were being taken. Figure 11 presents records of the interneuron HN(L,7) and of the excitor HE(L,7), which, according to the circuit diagram of Figure 4, receives inhibitory synaptic inputs from cells HN(3), HN(4) and HN(6). As can be seen, at the start of this record cell HN(L,7) began its active phase well in advance of the main ISP burst of cell HE(L,7), indicating that the left side was beating in the peristaltic mode, under which the cycles of cell HN(6) and HN(7) lead those of cells HN(3) and HN(4). During the later part of this record, however, the active phase of cell HN(L,7) coincides with the main ISP burst of cell HE(L,7), indicating that the left side was then beating in the non-peristaltic mode, under which the cycles of the four HN cells are nearly in phase. This record, therefore, supports the previous conclusion that the HN cells undergo shifts in the relative coordination of their activity cycles according to the two alternative coordination modes.

Unknown ISP Sources for HE Cells in Posterior Ganglia

For the anterior half of the ventral cord of the leech, the identification of the sources of HE cell ISP's is fairly complete. Apparently every HN cell that provides inhibitory synaptic input to the HE cells in ganglia 3 to 9 has been identified and, with the exception of cell HN(X), recorded from intracellularly. However, in the posterior half of the ventral cord, the survey is less complete and there occur HE cell ISP's whose sources are still unknown. For example, scrutiny of ISP bursts of cell HE(17) shows more ISP's than can be accounted for by impulses in the axons of the ipsilateral cells HN(6) and HN(7). Moreover, as can be seen in the records of Figure 12, although cells HE(R,17) and HE(R,18) do receive many of their ISP's from a common source, cell HE(R,18) also receives some ISP's which do not match any corresponding ISP's in the anterior HE(R,17) cell. As far as the HE(17) cell ISP's are concerned, it appears that some of them derive from a source which provides inhibitory synaptic input to both right and left members of the HE cell pair, since, as can be seen in the record of Figure 12, some of the ISP's in cell HE(R,17) can be matched

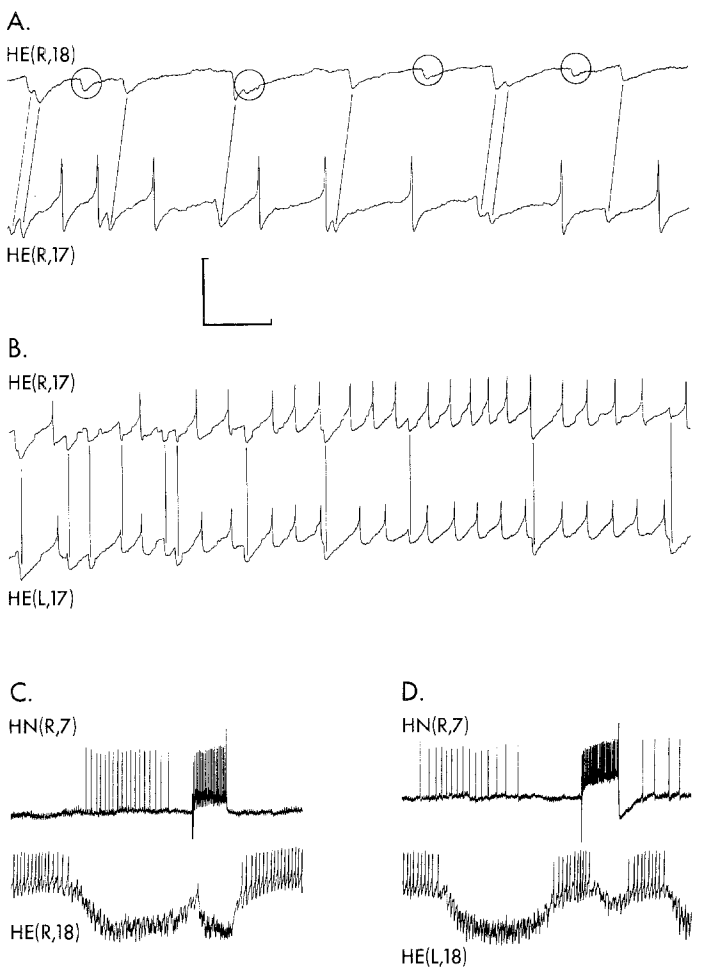

Fig. 12. Unidentified sources of ISP's for HE cells in posterior ganglia. Panel A: records show that some ISP's (indicated by circles) occur in cell HE(R,18) which cannot be matched with an ISP occurring in cell HE(R,17). Panel B: records show some matching ISP's in cells HE(R, 17) and HE(L,17). Panels C and D: passage of depolarizing current into cell HN(R,7) evokes a barrage of ISP's in both cell HE(R,18) and cell HE(L,18). Vertical calibration mark—panel A: HE(18) cell record: 7 mV; HE(17) cell record: 8 mV; panel B: both cells: 16 mV; panel C, HN cell record 20 mV; HE cell record: 15 mV; panel D: HN cell record: 20 mV; HE cell record: 18 mV. Horizontal calibration mark 250 ms in panel A; 500 ms in panel B; and 5 s in panels C and D

with synchronous ISP's in cell HE(L,17). No such bilateral match of HE cell ISP's has been found in any ganglion in the anterior half of the cord. And since the axon of cell HN(7) has been shown to form synapses only with the ipsilateral HE cells in posterior ganglia but not with the contralateral cell HE(18) (cf. Table 1), it can be inferred that the source of some of the HE(17) cell ISP's is another cell which makes connections with posterior HE cells. This cell, whose activity cycle would have to be in phase with that of cell HN(7), is, in fact, synaptically linked to it. As shown in Figure 12, passage

of depolarizing current into cell HN(R,7) evokes a burst of ISP's in both the ipsilateral and the contralateral cell HE(18). Although not shown in Figure 12, display of these bursts on an expanded time scale reveals that only some of the ISP's in the ipsilateral and none of the ISP's in the contralateral HE(18) cell can be accounted for by the impulses evoked in cell HN(R,7). It may be concluded, therefore, either that the axons of some HN cells previously identified in anterior ganglia make synaptic connections with posterior HE cells which escaped detection in the survey presented in Table 1, or that there exist additional, hitherto unidentified HN cells in posterior ganglia.

Heart Interneurons as Part of the Oscillator

We may now inquire whether the HN cells have their own endogenous activity rhythm—or possibly form part of an oscillatory neural circuit—or whether they are merely passively driven by another set of rhythmically active, hitherto unknown, neurons. The results of an experiment designed to answer this question are presented in Figure 13. As can be seen, passage of depolarizing current into cell HN(R,3) and evocation of a burst of action potentials during its inactive, or repolarized phase resets the rhythm of the heart interneuron. Whereas the HN cell action potential bursts prior to and after the evoked burst follow at nearly constant time intervals, the first HN cell action potential burst immediately following the evoked burst occurred after the normal interval reckoned from the evoked burst rather than from the last spontaneous burst. Tests of the susceptibility of the HN cell rhythm to resetting at different phases of its activity cycle showed that the cycle phase is reset only when an action potential burst is evoked during the inactive phase. Superposition of an evoked action potential burst and a spontaneous burst is without apparent effect on the phase of the next spontaneous burst. Resetting of the cycle phase does not occur, furthermore, upon passage of hyperpolarizing current into the cell during any phase, including the active phase when such passage can eliminate part or all of the spontaneous action potential burst.

A burst of action potentials evoked by passage of a pulse of depolarizing current into an HN cell resets the activity rhythm of not only that HN cell, but of the entire, compound ISP burst cycle in the HE cells. Hence, it appears that resetting of the phase of the rhythm of one heart interneuron resets also the rhythm of the other heart interneurons. These results thus indicate that the HN cells take part in the generation of their own activity rhythm. They are, therefore, active components of the neural oscillator that drives the leech heartbeat rhythm, rather than passively driven follower cells.

Multiple Sites of Rhythmic Impulse Burst Initiation in the Heart Interneurons

An important clue to the manner of function of the heart interneurons is provided by the finding that heart excitors such as cells HE(8), HE(9) and HE(10), which are known to receive their ISP inputs entirely from HN cells located

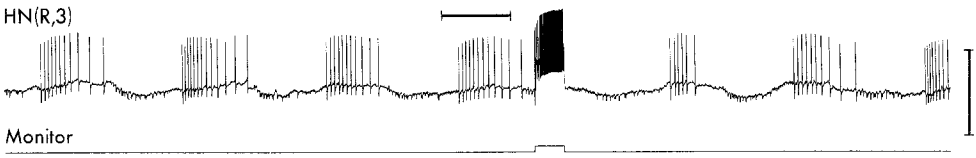

Fig. 13. Resetting of the HN cell rhythm by evoked action potentials. Brief passage of a depolarizing current pulse into the HN cell, marked by a deflection in the monitor trace, evokes a burst of action potentials which resets the HN cell rhythm. Vertical calibration mark – HN cell record: 40 mV. Horizontal calibration mark – 5 s

in anterior ganglia, and whose own ganglia do not seem to contain any HN cell bodies, nevertheless still show ISP bursts after complete isolation of their ganglia by scission of the anterior and posterior connectives. An explanation of this, on first sight surprising, finding is provided by data which show that although a neuronal process near the cell body is the normal site of impulse initiation in the heart interneuron, rhythmic impulse bursts can arise also in distant regions of the HN cell axon, one or more segments posterior to its ganglion of origin. Such multiple sites of impulse burst initiation have been reported previously in crustacean multisegmental interneurons (Kennedy and Mellon, 1964). The capacity of the HN cell for rhythmic impulse burst initiation in remote reaches of its axon was first seen when, upon passage of hyperpolarizing current into its cell body, the phasic activity changed from orthodromic to antidromic impulse bursts. That the antidromic impulse bursts observed under these conditions do, in fact, arise in posterior ganglia is shown by the records presented in Figure 14 of the concurrent activity of cell HN(L,4) and cell HE(L,5) in an isolated ventral cord preparation. The control record, taken while no current was passed into cell HN(L,4), confirms previous findings that each action potential in cell HN(L,4) precedes with constant lead time an ISP in cell HE(L,5). This order of precedence shows that the cell HN(L,4) impulses which produce cell HE(L,5) ISP's arise in ganglion 4 where the HN(L,4) cell body is located. In the test record, however, taken while hyperpolarizing current was passed into cell HN(L,4), the action potentials of cell HN(L,4) followed rather than preceded with constant lag time their matching ISP's in cell HE(L,5), indicating that here these presynaptic action potentials did not arise in ganglion 4 but in a ganglion posterior to it. Moreover, the action potentials recorded from the hyperpolarized HN cell can be seen to rise directly from the baseline without any prepotential, indicating that they are antidromic in character. This record also shows a number of instances in which the conduction of an antidromic action potential which caused an ISP in cell HE(L,5) failed at a site more remote from the HN(L,4) cell body than the normal site of conduction failure, and thus registered with a greatly reduced amplitude. The relative frequency of such antidromic action potential conduction failures rose with the strength of hyperpolarizing current passed into the HN cell body. Figure 14 presents also the results found upon passage of hyperpolarizing current into cell HN(R,3). As can be seen in the control record, each orthodromic action potential in cell HN(R,3) preceded with constant lead time an ISP in cell HE(R,7). However, upon hyperpolarization of cell HN(R,3), the ISP's in the heart excitor precede

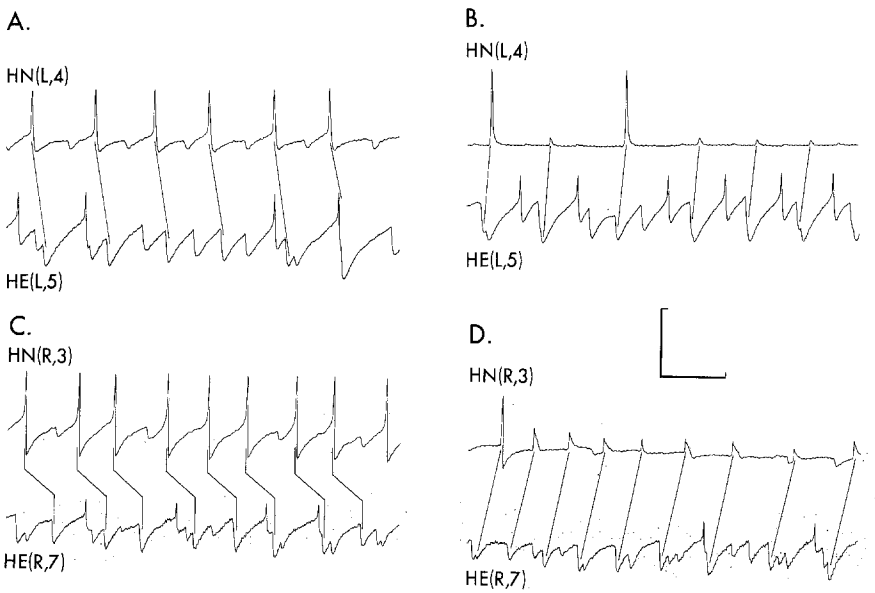

Fig. 14. Multiple sites of impulse initiation in the HN cells. Panels A and C: No polarizing current is passed into the HN cell, whose orthodromic action potentials precede matching ISP's in the HE cell. Panels B and D: Hyperpolarizing current is passed into the HN cell, whose antidromic action potentials follow matching ISP's in the HE cell. Vertical calibration mark – HN cell records: 55 mV; HE cell records: 18 mV. Horizontal calibration mark: 250 ms

the antidromic action potentials in the heart interneuron. Most of the antidromic action potentials seen in the test record are of small amplitude and hence their conduction failed at sites remote from the cell body. In addition, the delay of ISP's in cell HE(R,7) following orthodromic cell HN(R,3) action potentials was longer than the lead time with which they preceded the antidromic action potentials in the hyperpolarized interneuron. It can be inferred from this finding that the antidromic action potentials were initiated at a site located between ganglion 3 and ganglion 7.

A second indication that distant axonal regions of the HN cell axon are capable of initiating impulse bursts is provided by the occurrence of such bursts in HN cell axons that have been severed from their cell body. That such bursts occur can be inferred from records obtained from cell HN(L,4) in a completely isolated ganglion 4 while hyperpolarizing current was passed into the cell. Prior to hyperpolarization the heart interneuron of the isolated ganglion had shown bursts of action potentials as well as bursts of ESP's. Upon passage of hyperpolarizing current into cell HN(L,4), the action potential bursts ceased and no antidromic action potentials appeared, since the HN cell body had been severed from the remote impulse initiation sites of its axon in posterior ganglia. However, the bursts of ESP's persisted in the hyperpolarized cell. Since, as will be shown in the following paper (Thompson and Stent, 1976b), ESP's in cell HN(L,4) arise from impulses traveling in the axon of cell HN(L,3), their persistence in cell HN(L,4) of the isolated ganglion indicates that the axonal segment of cell HN(L,3) coursing through the isolated ganglion 4 continues to be rhythmically active in the absence of its cell body. It can be concluded therefore that

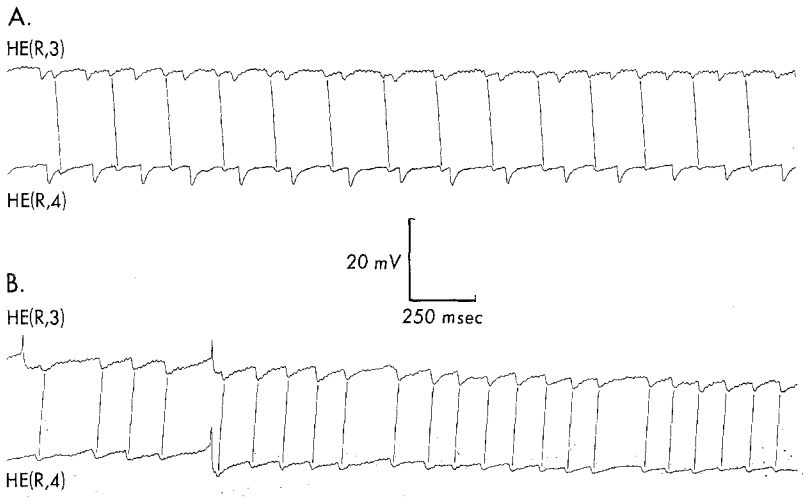

Fig.15. Rearward impulse conduction in the axon of the unidentified cell HN(X) under the peristaltic coordination mode and frontward impulse conduction under the non-peristaltic mode. The solid lines in the records taken from the two HE cells connect ISP's which are produced by impulses traveling in the axon of the HN(X) cell. Panel A: The right body side is coordinated in the peristaltic mode. Panel B: The right body side is coordinated in the non-peristaltic mode

the capacity of the heart interneurons to undergo an activity cycle that generates action potential bursts is not restricted to the normal impulse initiation sites in the ganglion in which the HN cell body is located but is present also at impulse initiation sites in remote sectors of the HN cell axons coursing through posterior ganglia of the ventral cord. Hence, the persistence of ISP bursts in HE cells of single isolated ganglia which do not contain any HN cell bodies is attributable to bursts of action potentials initiated within that ganglion in the severed HN cell axonal segments.

Detailed analysis of the order of precedence of matching ISP's recorded from pairs of homolateral HE cells has shown that for all known HN cells except HN(X), the direction of normal impulse conduction is always rearward in both the peristaltic and non-peristaltic coordination mode. This indicates that for each of the HN cells, except cell HN(X), the dominant site of impulse initiation is located in the ganglion containing the cell body. In the case of cell HN(X), however, the dominant impulse initiation site is located in different ganglia for the two coordination modes, as can be inferred from the data presented in Figure 15. The records taken from cells HE(R,3) and HE(R,4) during their peristaltic coordination mode show that ISP's due to cell HN(X) impulses appear first in cell HE(R,3) and later in cell HE(R,4). Thus, on the peristaltic body side the HN(X) impulses are initiated either in ganglion 3 or in ganglion 2. The records taken from the same two HE cells during the non-peristaltic coordination mode show a reversal of the ISP order of precedence, however. Evidently here the ISP's due to cell HN(X) impulses first appear in cell HE(R,4) and later in cell HE(R,3). Hence, the switch from the peristaltic to the non-peristaltic coordination mode is attended by a shift in the site of initiation of HN(X) impulses from ganglion 3 or a ganglion anterior

to ganglion 3 to ganglion 4 or a ganglion posterior to ganglion 4. Records taken from other HE cells indicate that the site at which HN(X) impulses are initiated during the non-peristaltic coordination mode is in fact posterior to ganglion 5. Inspection of the data of Figure 14 shows that the frequency of impulse initiation is higher at the posterior initiation site of cell HN(X), which is active in the non-peristaltic coordination mode, than at the anterior initiation site, which is active in the peristaltic coordination mode.

Discussion

The finding that the cycle of the heartbeat rhythm can be reset by evoking a premature HN cell impulse burst indicates that the HN cells form part of the neural oscillator that generates the heartbeat activity rhythm. There are two ways in which the HN cells might take part in the generation of their own activity rhythm. One is that they form part of a neuronal network whose activity oscillates because of the nature of its intercellular synaptic interactions (Wilson, 1966; Perkel and Mulloney, 1974; Selverston, 1974). Since, as will be shown in the next paper (Thompson and Stent, 1976b), HN cells do receive synaptic inputs from other HN cells, it is conceivable that the HN cell ensemble forms such an oscillatory synaptic network. The other main possibility is that each HN cell has the capacity for an endogeneous oscillation of its membrane potential (Alving, 1968; Mendelson, 1971; Maynard, 1972; Mayeri, 1973). In this case, the synaptic connections between the HN cells would serve only to coordinate, or phase-lock, their endogenous activity rhythms. A straightforward way of distinguishing between these two possible alternatives would be to determine whether the HN cell activity rhythm remains or disappears upon elimination of synaptic interactions among the HN cells. An attempt to perform this test was made in the present study. For this purpose chemical synaptic transmission between HN cells was abolished by bathing the preparation in Ca^{++}-free saline containing 20 mM Mg^{++}. It was found that under these conditions the HN cell impulse burst rhythm is *not* maintained; the cells enter a dormant, hyperpolarized state. Furthermore, passage of depolarizing current into such dormant HN cells gives rise only to tonic impulse activity and does not restore the impulse burst rhythm. However, this negative finding cannot be taken as conclusive evidence against the existence of an endogeneous activity rhythm of the HN cells, in view of recent results indicating that Ca^{++} is intimately involved in the generation of endogenous activity rhythms in molluscan oscillatory neurons (Eckert and Lux, 1976; Meech and Standen, 1975).

Indeed, on balance, the available evidence would seem to favor the endogeneous activity rhythm alternative. To reach this judgment we may consider the synaptic input pattern to an HN cell in an isolated ganglion. For instance, cell HN(4) in an isolated ganglion 4 continues to receive ESP bursts which are attributable to the rhythmic impulse activity of the HN(3) cell axon fragment in the neuropil of ganglion 4. Under these same conditions, cell HN(4) continues to receive also ISP bursts which, as will be shown in the next paper, are attributable mainly to the rhythmic impulse activity of its contralateral HN(4) cell homolog in the same ganglion. These synaptic input bursts continue unabated

even while hyperpolarizing current is being passed into the postsynaptic cell HN(4) and its own impulse bursts are held in abeyance. This finding indicates that the rhythmic impulse activity of cell HN(4) is not a necessary condition for the activity rhythms of the other HN cells that provide synaptic input to it. Furthermore, since under these conditions the ESP and ISP bursts in cell HN(4) maintain no fixed phase relation to each other, it can be inferred that the cell HN(3) axon fragment in ganglion 4 and the contralateral HN(4) cell do not depend on each other for the maintenance of their impulse rhythms. Unless, therefore, the individual HN cells, as well as their intersegmental axonal processes, undergo an endogenous activity rhythm, it would be necessary to envisage the presence of at least three separate network oscillators in ganglion 4: one in which the axon fragment of cell HN(3) takes part and two more in which each of the two HN(4) cells takes part. Moreover, since the impulse initiation sites in distant sectors of an HN cell axon are capable of rhythmic activity independent of that of the impulse initiation site in the ganglion in which the cell body is located, the synaptic interactions of the putative oscillatory network held responsible for the rhythm would have to be replicated in every ganglion. Thus, the most economical interpretation of these findings is to suppose that the HN cell has the capacity to undergo endogeneous oscillations of its membrane potential and that this capacity is widely distributed over its axons and dendrites. One impulse initiation site, in the case of cell HN(4) the one located in the ganglion of origin of the HN cell, would constitute the dominant oscillator of the cell, which would normally initiate the impulse burst, possibly because of a higher intrinsic frequency of impulse initiation. The impulses traveling rearward in the HN cell axon from the dominant oscillator could then lock the phase of the oscillations of the distant, or "recessive" axonal sectors in other ganglia. As envisaged by Stein (1974) for the coordination of the decapod swimmeret movement, this phase locking would occur because any phase drift between the cycles of the recessive and the dominant oscillator would be rectified by a delay of the phase of the next cycle of the recessive site, due to the arrival of impulses from the dominant site during its inactive phase.

In being subject to resetting of their cycle phase by evoked impulse bursts during their inactive phase, the HN cells resemble certain molluscan neurons known to be endogenous oscillators whose cycle can be similarly reset (Strumwasser, 1971; Kater and Kaneko, 1972; Kandel, 1967). That the HN cell cycle phase is not reset upon interruption of its normal impulse burst by passage of hyperpolarizing current into the cell during its active phase can be readily attributed to the fact that the independently active distant axonal sectors are not reached by current passed into the cell body and hence continue their normal rhythm. Moreover, the failure of an impulse burst evoked during the active phase of the HN cell cycle to reset the rhythm can be attributed to the reciprocal inhibitory synaptic connections which, as will be shown in the next paper, link individual HN cells to other rhythmically active HN cells. Thus, evoking additional impulses in an HN cell while that cell is inhibiting other HN cells is unlikely to affect the rhythm of those other cells, which will therefore preserve the original phase relations of the HN cell ensemble.

We may now consider, in view of the connections diagrammed in Figure 5, how the activity rhythm of the heart interneuron ensemble manages to generate the 180 ° frontward phase progression of the heart excitor activity cycles, which is, in turn, responsible for the frontward peristalsis of the heart tube on the peristaltic side. To simplify these considerations it shall be assumed that the activity rhythm of the HE cells is mainly determined by the periodic inhibitory input that these cells receive from their presynaptic HN cells and that any periodic excitatory input (which some HE cells are, in fact, known to receive via electrical junctions) makes only a minor contribution to the phasing the HE cell rhythm. Accordingly, the beginning or end of the HE cell impulse burst occurs when the periodic inhibitory input to the cell falls below or rises above the level necessary to maintain the HE cell membrane potential beneath its action potential threshold. Hence, the peristaltic frontward phase progression of the HE cell activity cycles is to be accounted for in terms of a systematic *blending* of the inhibitory synaptic output of the set of HN cells whose own activity rhythms maintain the frontward phase progression diagrammed in Figure 9.

In order to fathom the quantitative relations embodied by this synaptic blending process, we make the simplifying assumption that the amplitude a_{ij} of the periodic inhibitory input provided by a heart interneuron HN(i) to a heart excitor HE(j) varies with the phase angle x of the heartbeat cycle according to

$$a_{ij}=f_{ij}[\cos(x-g_i)+1]. \tag{1}$$

In this expression, f_{ij} is a parametric constant that expresses the relative strength of the inhibitory synaptic input provided by cell HN(i) to cell HE(j), where $\sum_i f_{ij}=1.0$, g_i is the constant phase lead that the cycle of cell HN(i) maintains relative to the overall beat cycle, and the amplitude a_{ij} is a measure of the total increase in polarizing membrane conductance at the impulse initiation site of cell HE(j) caused by the activity of cell HN(i). According to (1), the amplitude a_{ij} would vary between a minimum of 0 and a maximum of $2f_{ij}$ in the course of each beat cycle. Hence, the total amplitude of the periodic inhibition to which cell HE(j) would be subject is given by

$$A_j=\sum_i a_{ij}=\sum_i f_{ij}\,[\cos(x-g_i)+1]. \tag{2}$$

Since the sum of a set of cosine functions is itself a cosine function, we may write for the total periodic inhibition received by cell HE(j)

$$A_j=F_j\,[\cos(x-G_j)+1]. \tag{3}$$

Solution of Equation (2) and (3) for G_j, the phase lead of the periodic synaptic input to cell HE(j) relative to the overall beat cycle, yields

$$G_j=\tan^{-1}\left(\sum_i f_{ij}\sin g_i/\sum_i f_{ij}\cos g_i\right). \tag{4}$$

Thus the activity cycle of cell HE(j), as phased by this blend of inhibitory inputs, would occur in the corresponding antiphase, and be given by

$$P_j=180\,°+G_j=180\,°+\tan^{-1}\left(\sum_i f_{ij}\sin g_i/\sum_i f_{ij}\cos g_i\right). \tag{5}$$

Table 2. Relative phase relations of the HE cell activity cycles on the peristaltic body side calculated on the basis of Equation (5)

	Observed cycle phase	Order of calculation	Values of f_{ij} used in calculation					Calculated cycle phase P_j
			HN(X) $g_x=0°$	HN(3) $g_3=40°$	HN(4) $g_4=80°$	HN(6) $g_6=180°$	HN(7) $g_7=220°$	
HE(3)	200°	1st	0.50	0.50	0	0	0	200°
HE(4)	214°	1st	0.50	0.50	0	0	0	200°
		2nd	0.38	0.62	0	0	0	205°
HE(5)	218°	1st	0.33	0.33	0.33	0	0	220°
HE(6)	261°	1st	0.33	0.33	0.33	0	0	220°
		2nd	0.18	0.27	0.55	0	0	236°
HE(7)	315°	1st	0	0.33	0.33	0.33	0	268°
		2nd	0	0.11	0.16	0.73	0	293°
HE(9)	347°	1st	0	0.25	0.25	0.25	0.25	310°
HE(10)	359°	1st	0	0.25	0.25	0.25	0.25	310°
HE(13)		1st	0	0	0.33	0.33	0.33	348°
HE(17)	10°	1st	0	0	0	0.50	0.50	20°
HE(18)		1st	0	0	0	0.50	0.50	20°

Table 2 presents the results of calculations of the expected relative phase leads for the activity cycles of some of the HE cells on the peristaltic side, based on Equation (5), and the known phase leads maintained by their presynaptic HN cells. For the purpose of a first-order estimate it has been assumed that all HN cells which are known to contact a given cell HE(j), do so with equal synaptic strength, e.g., that for the synaptic input from cells HN(3), HN(4), HN(6) and HN(7) to cell HE(9), $f_{3,9}=f_{4,9}=f_{6,9}=f_{7,9}$. As can be seen from the data of Table 2, this first order calculation leads to HE cell phase lead values which approximate the observed phase relations on the peristaltic side. In agreement with observation, the cycles of the frontmost cell HE(3) and of the rearmost cell HE(18) are calculated to maintain a phase angle of 180 °, with most of this phase angle being accounted for by a frontward phase progression in the ganglia of the anterior body half. However, this first order calculation yields a saltatory rather than a smooth forward phase progression for the anterior body half, since according to it the cycles of cells HE(3) and HE(4) would occur in the same phase, as would those of cells HE(5) and HE(6). Furthermore, the estimated phase lead for the HE(7) cell cycle is too small. A second order calculation was therefore carried out for the cycles of cells HE(4), HE(6) and HE(7), in which the previous postulation of equality of the strengths of synaptic inputs from all pre-synaptic HE cells was abandoned, and a gradient of f_{ij} values was used. Under this gradient, in ganglia 4, 6, and 7 the rearmost HN cell provides the strongest and the frontmost HN cell the weakest synaptic input to the HE cell. The justification for this postulation, as well as the method by which the appropriate f_{ij} values were estimated, will be provided in the third paper of this series (Thompson and Stent, 1976b),

in terms of the presynaptic modulation which a posterior HN cell can exert on the synaptic efficacy of an anterior HN cell. The results of the second order calculation, presented also in Table 2, are evidently in quite good agreement with the observed phase progression of the HE cell activity cycles on the peristaltic side.

As far as the non-peristaltic side is concerned it is apparent that, in view of the nearly in-phase activity of all HN cells on that side, the HE cells whose inhibitory synaptic input is the result of a blending of the output of those HN cells will also maintain in-phase activity cycles, i.e., will command heart tube constrictions in concert rather than in a peristaltic mode. Cell HN(X) is, of course, an exception in that it maintains an activity cycle which is nearly antiphasic to those of the other HN cells on the non-peristaltic side. The influence of cell HN(X) on the most anterior HE cells on the non-peristaltic side can, therefore, be invoked to account for the slight antiperistaltic phase lead which the cycle of these cells maintains over the cycle of the posterior HE cells. The significance of cell HN(X) for the HE cell phase relations on the non-peristaltic side will be examined in more detail in the next paper.

Thus, it appears that the proposed blending of the inhibitory synaptic output of the set of HN cells can provide a reasonably quantitative account of the observed HE cell cycle phase relations on both peristaltic and non-peristaltic sides, based on the known pattern of HN-HE cell synaptic links and on the known relative phase relations of the HN cell activity cycles.

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