The Neural Control of Alternate Heartbeat Coordination States in the Leech, *Hirudo medicinalis*

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Summary. The bilateral paired hearts of the medicinal leech are controlled by a set of segmental heart motor neurons (HE cells) which are in turn controlled, via inhibitory synapses, by a set of segmental heart interneurons (HN cells). The HE cells fire in rhythmic impulse bursts because their steady discharge is periodically inhibited by the HN cells.

1) With the identification of an additional pair of HN cells in segmental ganglion I and the elucidation of several synaptic connections between HN cells a more complete heart control circuit diagram is now available. This circuit diagram accounts for the observed activity cycles of the various HN and HE cells and consequently for the behavior of the hearts themselves.

2) The bilaterally paired HE cells are coordinated by the HN cells such that the segmental heart tube sections on one side constrict in a caudorostral sequence to produce a rear-to-front peristalsis, while the segmental heart tube sections on the other side constrict nearly synchronously (non-peristaltically). This difference in the coordination modes of the two hearts is not permanent, and reciprocal coordination mode transitions occur every 10–50 heartbeat cycles. Cell HN(5) is phasically active on the side of the non-peristaltic heart tube and completely inactive on the side of the peristaltic heart tube. Reciprocal changes in the activity-inactivity pattern of the HN (5) cell pair are responsible for the observed spontaneous reciprocal changes in coordination mode. When cell HN (5) is made to be active or inactive, by intracellularly injected current, similar but unilateral changes, in coordination mode occur.

Introduction

Blood circulation in the vascular system of the leech, *Hirudo medicinalis*, is driven by the contractile rhythm of a bilateral pair of longitudinal coelomic...
Fig. 1 A and B. Peristalsis, non-peristalsis in the leech heart system. Panel A Schematic representation of the constriction times of the segmental heart tube sections during three heartbeat cycles illustrating their phase relations on the two body sides. On one side there is a definite rear to front progression in the constrictions of the segmental heart tube sections (peristalsis). On the other side the segmental heart tube sections constrict nearly in concert along the length of the heart tube (non-peristalsis). This panel is abstracted from Thompson and Stent (1976a). Panel B HE cell activity on the two body sides. The left side is coordinated peristaltically and the right side non-peristaltically.

vessels, the "heart tubes", which extend over the length of the body (Mann, 1962). The circular muscles located in the wall of the segmental heart tube sections (Hammersen and Staudte, 1969) undergo a constriction-dilation cycle whose period varies from 10 to 30 s, depending on the temperature and other, unidentified, factors. As a previous study (Thompson and Stent, 1976a) of the contractile rhythm, or "heartbeat," of these vessels has shown, the segmental heart tube sections on one side contract in caudostral sequence to produce a rear-to-front peristalsis, whereas the segmental heart tube sections on the other side contract nearly in concert, or non-peristaltically (Fig. 1A). The bilateral asymmetry of this constriction rhythm is not permanent however, in that there occur transitions after 10–50 heartbeat cycles in a given coordination mode. The result of such a transition is that the formerly peristaltic heart tube becomes coordinated in the non-peristaltic mode and the formerly non-peristaltic heart tube in the peristaltic mode. The constriction of each segmental heart tube section is controlled by a rhythmically active motor neuron, the heart excitor, or HE cell. A bilateral pair of HE cell bodies is located on the ventral aspect of each of the segmental ganglia 3 to 19 of the ventral
nerve cord (Fig. 2A), with each HE cell innervating via excitatory synapses the circular muscles of its ipsilateral segmental heart tube section. Figure 1B shows intercellular records taken from cells HE(L,3) and HE(R,3), i.e., of the heart motor neuron on the left and right sides, respectively, in ganglion 3. As can be seen, the HE cell activity cycle consists of an active phase, during which an impulse burst is produced and an inactive phase, during which there occurs a burst of inhibitory post-synaptic potentials, or IPSP's. In agreement with the constriction phase diagram of Figure 1A, the impulse bursts of the two contralateral HE cells of the third body segment—one active in the peristaltic and the other in the non-peristaltic mode—occur in antiphase. Moreover, the particular coordination mode of the whole heart tube is reflected by a caudorostral progression in which its ipsilateral HE cells begin their impulse burst during the heartbeat cycle (Thompson and Stent, 1976a).
The activity pattern of the HE cell ensemble is controlled by rhythmically active heart interneurons, or HN cells. Ganglia 3, 4, 6 and 7 of the nerve cord each contain a bilateral pair of HN cell bodies—cells HN(3), HN(4), HN(6), and HN(7). The HN cell body is also located on the ventral aspect of the segmental ganglion (Fig. 2A) and projects an axon rearward into the interganglionic connective, making inhibitory synaptic contacts with the ipsilateral HE cells in a series of posterior ganglia. The HN cells undergo an activity cycle which consists of an impulse burst and of an inactive phase during which there occurs a burst of IPSP’s. The impulse bursts of these four pairs of HN cells can account for all of the rhythmic inhibitory input received by all the HE cells, except for a class of IPSP’s common to cells HE(3), HE(4), HE(5) and HE(6) which has been attributed to an unidentified heart interneuron, cell HN(X). The manner in which the HN cells are connected to the HE cells is shown in the hemilateral circuit diagram of Figure 2B (Thompson and Stent, 1976b). Since the direction of interganglionic travel of the cell HN(X) impulses differs according to the heartbeat coordination mode—rearward on the peristaltic and frontward on the non-peristaltic side—it is possible that there exist not one but two HN(X) cells on each side, of which one is active only in the peristaltic and the other only in the non-peristaltic mode. However, for the sake of simplicity it will be assumed in this paper that there is only one HN(X) cell on each side, whose axon possesses an anterior impulse initiation site, HN(X)ₐ active only in the peristaltic mode and a posterior initiation site, HN(X)ₚ active only in the non-peristaltic mode.

In addition to these HN cells, two further pairs of rhythmically active interneurons, cells HN(2) and HN(5), have been identified which provide synaptic input to other HN cells but not to HE cells (Thompson and Stent, 1976c).

The motor neuron and interneuron activity pattern responsible for the heartbeat is generated wholly within the central nervous system, since the normal rhythm of the entire neuronal ensemble, including right-left transitions between peristaltic and non-peristaltic coordination modes, occurs in isolated leech nerve cord preparations deprived of all sensory input. Moreover, all the available evidence supports the notion that the HN cells (except HN(5)) produce their rhythmic impulse bursts, and hence the heart beat rhythm, due to endogenous membrane properties (Thompson and Stent, 1976c; Calabrese, in preparation). Each ganglionic section of an HN cell (except HN(5)) is capable of expressing this endogenous impulse rhythm in isolation but normally the impulse initiation site in the ganglion of origin of the cell is dominant over and suppresses the rest (Thompson and Stent, 1976c). The HE cells however, would be tonically active were it not for the rhythmic inhibition they receive from the HN cells that drives them into impulse bursts (Calabrese, in preparation).

Materials and Methods

Specimens of Hirudo medicinalis were purchased from a commercial supplier and maintained before use in aquaria at 15°C for periods up to six months. All experiments were carried out with isolated leech nerve cord preparations, consisting of the head brain and at least the first 8 segmental
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ganglia (unless specified otherwise in the text). The experimental methods of maintaining the preparations and of obtaining intracellular electrophysiological records from and passing current into leech neurons were those previously described (Kristan et al., 1974; Thompson and Stent, 1976a). Within a given segmental ganglion HE and HN cells were identified by the position of their cell bodies, by their characteristic impulse burst pattern with a period equal to that of the heartbeat, and by their synaptic connections with other neurons of the heartbeat control system. Two such neurons were judged to be synaptically connected if individual impulses recorded from one neuron’s cell body were followed with constant delay (or in some cases, preceded with constant lead time) by a post-synaptic potential in the other neuron.

Results

Heart Interneurons HN(1) and HN(2)

A previously undiscovered heart interneuron HN(1) was identified on both sides of the first ganglion of the ventral nerve cord. As shown in the records of Figure 3 A, it is possible to record rhythmic impulse bursts from the HN(1)
cell body. The monophasic character and the attenuated amplitude of the impulses seen in cell HN(1) indicate that they are antidromic spikes. No synaptic potentials are manifest in records taken from the HN(1) cell body, and cell HN(1) does not form any synaptic connections with either ipsilateral or contralateral heart motor neurons. Thus cell HN(1) closely resembles cell HN(3), which similarly shows bursts of antidromic spikes (Fig. 4A) and does not connect to any HE cells (Thompson and Stent, 1976c). It is possible to evoke orthodromic spikes in both cells HN(1) and HN(2) by passage of large (>10 nA) depolarizing currents into their cell bodies. The resulting spikes are of very low amplitude (<0.5 mV) and are usually hidden in the electrode noise attending intracellular passage of large currents. Nevertheless, the synaptic effects of these evoked impulses can be registered in HN cells of posterior ganglia.

As can be seen in Figures 3B and D, each impulse recorded from the HN(1) cell body was preceded with constant lead times of 24 ms and 40 ms by an IPSP in the ipsilateral cells HN(3) and HN(4), respectively. Hence, HN(1) forms an inhibitory synapse with the ipsilateral cells HN(3) and HN(4). The finding that the IPSP's recorded in cells HN(3) and HN(4) precede the impulse recorded in the HN(1) cell body indicate that these impulses are initiated in a remote part of the HN(1) cell which is closer to ganglion 3 and 4 than to ganglion 1.

The records of Figure 4B show that each impulse recorded from the HN(2) cell body is preceded by an IPSP in the ipsilateral cell HN(4). Since it had been previously shown that each HN(2) impulse is preceded by an IPSP in the ipsilateral cell HN(3) (Thompson and Stent, 1976c), it can be concluded that cell HN(2), just as cell HN(1), forms an inhibitory synapse with the ipsilateral cells HN(3) and HN(4) and that its impulses arise at an impulse initiation site posterior to ganglion 2.
As can be seen in the records of Figures 3A, 3C and 4A (as well as of the later Figure 12) the impulse bursts of cells HN(1) and HN(2) occur in antiphase with the impulse bursts of the ipsilateral cells HN(3) and HN(4). It follows, therefore, that the impulse bursts of the ipsilateral cells HN(1) and HN(2) occur in phase with one another. Moreover, since the impulse bursts of cells HN(3) and HN(4) on one side occur in antiphase with those of their contralateral homologs (Thompson and Stent, 1976 b), it follows that the impulse bursts of cells HN(1) and HN(2) on one side also occur in antiphase with those of their contralateral homologs.

Heart Interneurons HN(3) and HN(4)

The synaptic connections and activity cycle phase relations of the heart interneurons HN(3) and HN(4) had been ascertained previously, either by the direct method of recording from the interneurons themselves or by the indirect method of recording from their postsynaptic cells and matching postsynaptic potentials. Thus it had been found that cells HN(3) and HN(4) form inhibitory connections with their respective contralateral homologs and with the ipsilateral cell HN(5). Moreover, cell HN(3) forms an electrical junction with the ipsilateral cell HN(4), and both cell HN(3) and cell HN(4) form electrical junctions with the ipsilateral cells HN(6) and HN(7) (Thompson and Stent, 1976 b, c). These findings were confirmed in the present study. For instance, the data of Figures 5A and C confirm by the direct method of matching presynaptic impulses with postsynaptic IPSP's the inhibitory link from cell HN(3) to the ipsilateral cell HN(5), which had been previously shown only by the indirect method. These data confirm also the previous indirect inference that the activity cycles of the ipsilateral cells HN(3) and HN(5) occur in antiphase. Similarly, the data of Figures 5C and D demonstrate by the direct method the previously inferred electrical junction between the cell HN(3) and the ipsilateral cells HN(6) and HN(7).

Heart Interneuron HN(5)

Cell HN(5) had previously been shown by the direct method to form an inhibitory connection with the contralateral cell HN(7) and by the indirect method to form such a connection with the ipsilateral cell HN(7). The records of Figure 6 confirm the existence of these connections by the direct method and demonstrate also a previously unknown inhibitory link from cell HN(5) to both the ipsilateral and the contralateral cell HN(6).

If the bilateral HN(5) cell pair represents the only source of inhibitory synaptic input to the bilateral HN(6) and HN(7) cell pairs, then all IPSP's recorded from these four cells should match. The data of Figure 7 show that this is, in fact, the case. Moreover, since, as was previously inferred (Thompson and Stent, 1976 a) only the HN(5) cell on the non-peristaltic side is normally active, with the HN(5) cell on the peristaltic side being completely inactive, only a single class of IPSP's is observed in the HN(6) and HN(7) cell pairs.
Fig. 5 A–D. Connections and phase relations of cell HN(3). Panel A Recordings from ipsilateral cells HN(3) and HN(5) showing that their impulse burst rhythms are antiphase. Panel B Each action potential recorded in the HN(R,3) cell body is followed at a constant delay by an IPSP recorded in the HN(R,5) cell body, demonstrating an inhibitory synaptic connection from cell HN(3) to ipsilateral cell HN(5). The delay in these records is so small because all the cell HN(R,3) action potentials are being abnormally initiated in ganglion 4 due to a penetration injury which rendered cell HN(R,3) incapable of initiating action potentials in ganglion 3, unless depolarized by passage of large currents.) Panel C Ten superimposed oscilloscope sweeps triggered by action potentials recorded in the HN(L,3) cell body which are followed at a constant delay by EPSP's recorded in the HN(L,6) cell body, demonstrating an excitatory synaptic connection from cell HN(3) to ipsilateral cell HN(6). Panel D Eleven superimposed oscilloscope sweeps triggered by action potentials recorded in the HN(R,3) cell body which are followed at a constant delay by EPSP's recorded in the HN(R,7) cell body, demonstrating an excitatory synaptic connection from cell HN(3) to ipsilateral cell HN(7). The EPSP's shown in panels C and D are electrical since they persist in saline which contains 20 mM Mg++. Such saline is known to block chemical, but not electrical, synaptic transmission in the leech (Nicholls and Purves; 1970)

(Figs. 6 A, 7 B, 8 A) and all of these IPSP's can be accounted for by impulses recorded from the active HN(5) cell (Fig. 6A). Accordingly, the impulse burst rhythm of the active HN(5) cell occurs in antiphase with that of the HN(6) and HN(7) cell pairs (Fig. 6A). However, under some experimentally imposed abnormal conditions an impulse burst rhythm may occur in both members of the HN(5) cell pair, in which case two classes of IPSP's can be observed in the HN(6) and HN(7) cell pairs (Figs. 8B and 16B).
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Fig. 6 A–E. Connections and phase relations of cell HN(5). Panel A Recordings from ipsilateral cells HN(5) and HN(7) show that their impulse burst rhythms are antiphase in the non-peristaltic coordination mode. (All members of the HN(6) and HN(7) cell pairs are similarly phased with an active cell HN(5). Panels B–E In each case there are superimposed oscilloscope sweeps triggered by action potentials recorded in a particular HN(5) cell body which are followed at a constant delay by IPSP's recorded in the cell body of a particular member of the HN(6) or HN(7) cell pairs. These records demonstrate an inhibitory synaptic connection from cell HN(5) to both ipsilateral and contralateral cells HN(6) and HN(7). Eight, greater than twenty, fifteen, and fifteen superimposed sweeps in panels B–E respectively.

Fig. 7 A and B. Matching of IPSP's and EPSP's in the HN(6)–HN(7) cell group and phase relations within the group. Panel A Recordings from ipsilateral cells HN(6) and HN(7) showing that their impulse burst rhythms are in phase. (All members within the group are similarly phased with respect to one another.) Panel B Matching of a single class of IPSP's and multiple EPSP's in ipsilateral cells HN(6) and HN(7). Solid lines indicate matched IPSP's and dashed lines indicate matched EPSP's. The HN(7) spikes are clipped by the pen-writer in B.
No indication has been found thus far that the heart interneurons HN(6) and HN(7) form synaptic connections with other HN cells. The data of Figures 7 and 8 show, moreover, that these cells do not form any inhibitory connections or electrical junctions among themselves, since all IPSP's in these records are accountable by synaptic input from cell HN(5) and no EPSP's can be accounted for by impulses within the group. These data show also the occurrence of common excitatory postsynaptic potentials (EPSP's) in the ipsilateral (but not in the contralateral) HN(6) and HN(7) cells, in accord with the previous finding of electrical junctions between cells HN(3) and HN(4) and ipsilateral cells HN(6) and HN(7). As can be inferred from the records presented in Figures 7 and 8, the impulse burst rhythms of all four members of the HN(6) and HN(7) cell pairs occur in approximately the same phase.

The Unidentified Heart Interneuron HN(X)

By matching IPSP's observed in the motor neuron HE(3) attributable to impulses in the axon of cell HN(X) with EPSP's observed in other HN cells it had been previously shown that the axon of HN(X) forms electrical junctions with the ipsilateral (but not with the contralateral) cells HN(3) and HN(4). The existence of these junctions, as well as of all other known connections of cell
HN(X) has been confirmed in the present study under both the peristaltic and the non-peristaltic coordination modes. (Hence, as mentioned in the Introduction, if cell HN(X) should turn out to represent two separate cells, then both cells would have to have identical synaptic connection patterns.) Despite an intensive search, no additional connections of cell HN(X) have been found in this study. In particular, this search has shown that cell HN(X) does not form any electrical junctions with ipsilateral cells HN(5), HN(6) and HN(7) as it does with ipsilateral cells HN(3) and HN(4).

The Heart Interneuron Connectivity Pattern

The connections among the heart interneurons identified thus far are summarized in Figure 9A and in Table 1. No other heart interneurons have been found, despite a systematic survey of the cell bodies in the lateral cell packets of the ventral aspect of ganglia 1 to 8. How complete is this neuronal circuit diagram? Inasmuch as no synaptic potentials can be recorded from the HN(1) and HN(2) cell bodies, the nature of their synaptic input remains in doubt. However, since the activity cycles of cells HN(1) and HN(2) on one side are synchronous with each other and antiphasic with their contralateral homologs, it seems likely that (in homology with the known connections of cells HN(3) and HN(4)) reciprocal inhibitory synapses link contralateral HN(1) and HN(2) homologs as shown in Figure 9B and an electrical junction links the ipsilateral cells HN(1) and HN(2). However, all of the observed classes of synaptic potentials, except one, can be accounted for by the circuit diagram of Figure 9A. For example, as shown by the records of Figures 7 and 8, all of the synaptic potentials observed in cells HN(6) and HN(7) can be accounted for by their known connections with cells HN(3), HN(4) and HN(3). The one unaccounted for class of synaptic potentials consists of EPSP's observed in cell HN(5). As shown in Figure 11A, passage of hyperpolarizing current into cell HN(5) during its impulse burst reveals the presence of a burst of normally masked small EPSP's. The source of these EPSP's appears to be an axon coursing in the ipsilateral interganglionic connective through ganglion 5. The data of Figure 11B show that an electrical stimulus delivered to the ipsilateral 3–4 interganglionic connective gives rise to an EPSP in the HN(5) cell after a constant delay of 20 ms. However, similar stimulation of the contralateral 3–4 connective did not elicit an EPSP. Moreover, a stimulus delivered bilaterally to the 6–7 interganglionic connective (in which the rearward-going axon of cell HN(5) is present) gives rise to an EPSP in that HN(5) (20 ms delay) cell which is, in turn, followed by an antidromic spike (55 ms delay). Hence the unidentified axon that is the source of the EPSP's appears to have a higher impulse conduction velocity than the axon of cell HN(5).

Phase Relations of the Heart Interneuron Activity Cycles

Figure 10 presents an extension of the previously published phase diagram of the activity cycles of the heart interneurons HN(X), HN(3), HN(4), HN(6)
Fig. 9A and B. Panel A Schematic circuit diagram showing known synaptic connections among heart interneurons. The labeled circles represent a particular nerve cell's "dominant" impulse initiation site which is usually located in the ganglion where the cell body of that interneuron is located. The lines represent cell processes. Panel B Simplified schematic circuit diagram of the HN cell synaptic interactions including hypothesized synaptic connections and assignment of the approximate phase angle at the start of their impulse burst for each HN cell. The labeled circles represent a particular nerve cell's "dominant" impulse initiation site and the lines cell processes (dashed lines for hypothesized connections). HN cells with similar input and/or connections are grouped together. Only inhibitory connections (except the excitatory connection from cell HN(5) to the ipsilateral HN(X), impulse initiation site) are shown. "Fixed-phase" interneurons are assigned a single approximate phase angle while "variable-phase" cells are assigned two approximate phase angles. Cells HN(5) and HN(X) are assigned an approximate phase angle for their phasically active state and "off" for their completely inactive state. Where there are dual phase angle assignments the one to the left of the slashed line pertains when the right side is in the peristaltic coordination mode and the one to the right of the slashed line pertains when the right side is in the non-peristaltic coordination mode.
Table 1. Synaptic connections between HN cells. Each entry represents the result of simultaneous intracellular recordings from the cells indicated. Only HN cell pairs not previously recorded by Thompson and Stent (1976a) are listed.

<table>
<thead>
<tr>
<th>From cell*</th>
<th>To cell</th>
<th>Type of connection</th>
<th>Delay between action potential of HN cell listed in column 1 and the post-synaptic potential in the HN cell listed in column 2 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN(L,1)</td>
<td>HN(L,3)</td>
<td>Inhibitory</td>
<td>-24</td>
</tr>
<tr>
<td>HN(L,1)</td>
<td>HN(L,4)</td>
<td>Inhibitory</td>
<td>-40</td>
</tr>
<tr>
<td>HN(L,2)</td>
<td>HN(L,4)</td>
<td>Inhibitory</td>
<td>-8</td>
</tr>
<tr>
<td>HN(R,2)</td>
<td>HN(R,4)</td>
<td>Inhibitory</td>
<td>-40</td>
</tr>
<tr>
<td>HN(R,3)</td>
<td>HN(R,4)</td>
<td>Inhibitory</td>
<td>8</td>
</tr>
<tr>
<td>HN(L,3)</td>
<td>HN(L,5)</td>
<td>Inhibitory</td>
<td>0*</td>
</tr>
<tr>
<td>HN(L,3)</td>
<td>HN(L,6)</td>
<td>Excitatory</td>
<td>110</td>
</tr>
<tr>
<td>HN(R,3)</td>
<td>HN(R,7)</td>
<td>Excitatory</td>
<td>100</td>
</tr>
<tr>
<td>HN(R,5)</td>
<td>HN(R,6)</td>
<td>Inhibitory</td>
<td>60</td>
</tr>
<tr>
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<td>HN(L,6)</td>
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<td>45</td>
</tr>
<tr>
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<td>HN(R,7)</td>
<td>Inhibitory</td>
<td>80</td>
</tr>
<tr>
<td>HN(R,5)</td>
<td>HN(R,7)</td>
<td>Inhibitory</td>
<td>150</td>
</tr>
<tr>
<td>HN(R,6)</td>
<td>HN(R,7)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>HN(L,6)</td>
<td>HN(R,6)</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>HN(R,6)</td>
<td>HN(L,6)</td>
<td>None</td>
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</tr>
<tr>
<td>HN(R,6)</td>
<td>HN(L,7)</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

* In each pairwise recording synaptic connections in both directions were tested for

* Cell HN(R,3) was abnormally initiating its impulses in ganglion four which resulted in the latency of 0 ms from an impulse in the HN(R,3) cell body to an IPSP in cell HN(R,5)

Right body side (peristaltic)

HN(L)  HN(1)  HN(2)  HN(3)  HN(4)  HN(5)  HN(6)  HN(7)

HN(L)  HN(1)  HN(2)  HN(3)  HN(4)  HN(5)  HN(6)  HN(7)

Left body side (non-peristaltic)

HN(X)  HN(1)  HN(2)  HN(3)  HN(4)  HN(5)  HN(6)  HN(7)

HN(X)  HN(1)  HN(2)  HN(3)  HN(4)  HN(5)  HN(6)  HN(7)

Fig. 10. Phase diagram of HN cell impulse burst activity with the right side in the peristaltic coordination mode and the left side in the non-peristaltic coordination mode. The bars indicate the duration of an HN cell's impulse burst activity. The lack of a bar indicates that the cell is inactive. This diagram was generated from pairwise recordings of HN and HE cells. Cell HN(R,1) was arbitrarily assigned a phase angle of 0° at the start of its impulse burst.
Fig. 11 A and B. Excitatory synaptic inputs to cell HN(5). Panel A Hyperpolarization of cell HN(5) during its impulse burst reveals underlying EPSP's for the next few heartbeat cycles. The bottom trace is a high gain AC coupled version of the corresponding portion of the top trace. Arrows indicate EPSP's. Panel B 1) Electrical stimulation of the ipsilateral 3-4 interganglionic connective elicits an EPSP in cell HN(5) (arrow). 2) Electrical stimulation of the contralateral 3-4 interganglionic connective has no effect on cell HN(5). 3) Electrical stimulation of both 6-7 interganglionic connectives elicits, with a short delay, an EPSP (first arrow) and with long delay an antidromic action potential in cell HN(5) (second arrow). In 1) and 2) there are ten, and in 3) there are twenty, superimposed oscilloscope sweeps which were triggered from the stimulus artifact. The stimulus rate was 1 Hz in 1) and 2) and 10 Hz in 3).

and HN(7) (Thompson and Stent, 1976b). The present diagram includes in addition the phases of the activity cycles of cells HN(1), HN(2) and HN(5), as determined by comparing records of their impulse burst rhythms with records taken simultaneously from HE cells in both peristaltic and non-peristaltic coordination modes. In the construction of this phase diagram the convention has been adopted of arbitrarily assigning the phase angle 0° to the start of the impulse burst in the activity cycle of cell HN(R,1), regardless of whether the right side is coordinated in the peristaltic or non-peristaltic mode. This convention differs from that adopted by Thompson and Stent (1976b) who assigned the phase angle 0° to the start of the impulse burst in cell HN(X) in the peristaltic coordination mode. The present convention appears to be preferable because it reflects the fact, to be elaborated in the following, that the absolute phase angles of the activity cycles of heart interneurons HN(1), HN(2), HN(3) and HN(4) are independent of the heartbeat coordination mode, whereas the absolute phase angles of the activity cycles of cell HN(X) and of interneurons HN(6) and HN(7) change with the coordination mode.

The diagram of Figure 10 presents the situation that obtains when the right side is coordinated in the peristaltic coordination mode. As can be seen, on that right side the activity cycles of the cells form a more or less continuous phase progression, in the sequence HN(R,1) and HN(R,2) at 0°, HN(R,7)
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at 60°, HN(R,6) at 80°, HN(R,4) at 180°, HN(R,3) at 220°, and HN(R,X) at 260°, with cell HN(R,5) being inactive. By contrast, on the non-peristaltic left side, the interneuron activity cycles form an ordinarily different phase progression, in the sequence HN(L,4) at 0°, HN(L,3) at 40°, HN(L,7) at 60°, HN(L,6) at 80°, HN(L,1) and HN(L,2) at 180°, and HN(L,X) and HN(L,5) at 220°.

How does this phase diagram change upon a right-left switch of the coordination mode? First, the previously inactive cell HN(R,5) becomes active in the phase 40°, and the previously active cell HN(L,5) becomes inactive. Consequently, the bilateral cell pairs HN(6) and HN(7) are now inhibited by HN(R,5) instead of by HN(L,5) and hence shift their activity phase by 180°. For reasons that will be considered later, the bilateral cell pair HN(X) also shifts its activity phase by 180°. However, the activity cycles of the bilateral cell pairs HN(1), HN(2), HN(3) and HN(4) would be unaffected by this change in coordination mode and continue in their previous phase. In view of these facts, cells HN(1), HN(2), HN(3) and HN(4) will be referred to as “fixed-phase interneurons” and cells HN(6) and HN(7) will be referred to as “variable-phase interneurons”. Cells HN(5) and HN(X) are special cases and are not included in either of these classes.

Figure 9B presents a schematic summary of established and hypothesized inhibitory synaptic connections between the interneurons and their phase relations under both coordination modes. (The excitatory synaptic connections have been eliminated because they are not as important in the phasing of HN cells as are the inhibitory connections.) In this diagram, interneurons with similar output and input connections have been grouped together. A single approximate phase angle has been indicated for each fixed-phase interneuron; two phase angles have been indicated for each variable-phase interneuron; and the possibility of being either active in a given phase or inactive has been indicated for cell HN(5) and for the anterior and posterior HN(X) impulse initiation sites HN(X)a and HN(X)p. It is evident that the connections shown in Figure 9B readily account for the observations that the contralateral homologs of cells HN(1), HN(2), HN(3) and HN(4) are active in antiphase, that cells HN(1) and HN(2) are active roughly in antiphase with the ipsilateral cells HN(3) and HN(4), that when cell HN(5) is active it is active roughly in antiphase with the ipsilateral cells HN(3) and HN(4) and with the bilateral cell pairs HN(6) and HN(7). Furthermore, if it is assumed that the HN(X)a impulse initiation site does not produce impulse bursts endogenously and instead is driven by excitatory synaptic input from the ipsilateral cell HN(5) (cf., Fig. 9B), the connections shown in the diagram also explain why HN(X)p is inactive on the peristaltic side and active on the non-peristaltic side in phase with the ipsilateral cell HN(5). The connections shown in the diagram do not, however, provide the answers to four important questions:

1. How is cell HN(5) held inactive on the peristaltic side?
2. How is the HN(X)a impulse initiation site held inactive on the peristaltic side?
3. How is the right-left transition in coordination mode effected?
4. How are the small but significant ipsilateral phase leads in the activity cycles of HN(4) over HN(3), of HN(3) over HN(X) and of HN(7) over HN(6) generated?
Spontaneous Transitions of the Coordination Mode

To find experimental answers to the first three of these questions, it is useful to be able to determine from the intracellular record of a single cell whether a given side is coordinated in the peristaltic or non-peristaltic heartbeat mode. Such a determination can in fact, be made on the basis of records taken from single HE and HN cells of the 3rd and 4th ganglion. For instance, the records of Figure 1B show that the two contralateral HE(3) cells receive a different pattern of IPSP inputs: in cell HE(L,3) the two classes of large amplitude and small amplitude IPSP’s occur serially, whereas in cell HE (R, 3) they occur concurrently. The first of these IPSP patterns has been shown to be characteristic of the peristaltic and the second characteristic of the non-peristaltic coordination mode (Thompson and Stent, 1976c).

Figure 12 presents analyses of the composition of IPSP bursts in cell HE(L,3) when the left side was first in the peristaltic and later in the non-peristaltic coordination mode. While the left side was coordinated in the peristaltic coordination mode (Fig. 12A) the HE(L,3) impulse burst terminated at the start of the HN(L,3) impulse burst. Under these conditions, the class of large amplitude IPSP’s observed in cell HE(L,3) was completely matched by (and hence attributable to) impulses in cell HN(L,3) and the class of small amplitude IPSP’s, concurrent with the large amplitude IPSP class, was matched by EPSP’s in cell HN(L,3) during its active phase, and hence attributable to impulses in cell HN(L,3) (Fig. 12B). Upon the switch of the left side to the non-peristaltic coordination mode (Fig. 12C) the HE(L,3) impulse burst became nearly concurrent with the HN(L,3) impulse burst. Under these conditions, the two classes of large and small amplitude IPSP’s occurred sequentially in cell HE(L,3) (Fig. 12D). The class of small amplitude IPSP’s, which were matched by EPSP’s in cell HN(L,3) during its inactive phase and hence attributable to impulses in cell HN(L,3), evidently terminated the HE(L,3) impulse burst. This IPSP class was followed by the class of large amplitude IPSP’s which were matched by (and hence attributable to) impulses in cell HN(L,3), that occurred during the HE(L,3) impulse burst. Hence, it is possible to determine the coordination mode from the record taken from a single HE(3) cell: if the small and large amplitude IPSP’s occur concurrently then the side of the cell is coordinated in the peristaltic mode; but if the small amplitude IPSP’s precede the large amplitude IPSP’s then that side is in the non-peristaltic mode. The same diagnostic criteria for the coordination mode apply also to records taken from cell HE(4), whose IPSP’s are derived from the same presynaptic sources as those of cell HE(3). Similarly, the coordination mode can be inferred from the record taken from a single HN(3) cell: if the EPSP’s (due to HN(3) impulses) occurred in cell HN(3) during its active phase, then the side of that cell was coordinated in the peristaltic mode; but if the EPSP’s in cell HN(3) occurred during its inactive phase, then the side was coordinated in the non-peristaltic mode.

Analogous diagnostic criteria can be applied also to records taken from the motoneurons HE(5) and HE(6), and from the interneuron HN(4). Cells HE(5) and HE(6) differ from cells HE(3) and HE(4) in that they receive another class of large-amplitude IPSP’s, namely those provided by the ipsilateral cell HN(4). However, regardless of the coordination mode, the large-amplitude IPSP’s derived from cell HN(4) always occur very nearly in phase with the large-amplitude IPSP’s derived from cell HN(3). Hence, the peristaltic coordination mode is indicated if in a record taken from cell HE(5) or cell HE(6) the small-amplitude class of IPSP’s derived from cell HN(4) occurred concurrently with the large-amplitude class of IPSP’s derived from cells HN(3) and HN(4). By contrast, the non-peristaltic mode is indicated if in such a record the small-amplitude IPSP’s preceded the large-amplitude IPSP’s. Similarly, cell HN(4) differs from cell HN(3) in that it receives an additional class of EPSP’s, namely that derived from the ipsilateral cell HN(3). However, regardless of the coordination mode, these additional EPSP’s always occur during the active phase of cell HN(4). Hence the peristaltic coordination mode is indicated if in a record taken from cell HN(4) all EPSP’s occurred during the active phase of the cell. By contrast, the non-peristaltic mode is indicated if some of the EPSP’s (namely those derived from the ipsilateral cell HN(3)) occurred during the inactive phase of cell HN(4).
By applying these diagnostic criteria to a large number of paired records taken simultaneously from two heartbeat control neurons in the first seven ganglia of an isolated nerve cord (e.g., Fig. 13), it can be concluded that all ipsilateral motor neurons and interneurons are always coordinated in the same mode and that if the neurons of one side are coordinated in the peristaltic mode then the neurons of the other side are always coordinated in the non-peristaltic mode. Moreover, as can be seen in the records taken from the HE (4)
cell pair presented in Figure 13A, the transition of one side from one coordination mode to the other is always accompanied by a reciprocal transition in coordination mode of the other side and the records taken from HE(R,3) and HE(R,4) presented in Figure 13B indicate that all the heartbeat control neurons on a particular side undergo a coordination mode transition in concert. The complete reciprocal transition in coordination mode may be accomplished in a single heartbeat cycle (as in the record of Fig. 13B) or it may extend over two or more heartbeat cycles (as in the record of Fig. 13A). In both cases, the transition always reflects a change in the phase of the impulse burst activity of cell HN(X), as judged by the occurrence of the small-amplitude IPSP’s attributable to it in cells HE(3) and HE(4): Cell HN(X) either fails to produce an impulse burst at the expected time and then resumes its burst activity in a new phase relative to the activity cycle of cell HN(3), or it produces an early second impulse burst within a single heartbeat cycle and then continues to produce regular impulse bursts in the new phase relation indicated by the early burst. As can be seen in the records of Figure 13, the impulse burst rhythm of HN(3), as reflected by the occurrence of large amplitude IPSP’s in cells HE(3) and HE(4), remains unaltered during the transitions in coordination mode and changes in the phase of the cell HN(X) activity cycle.

The records of Figure 14 show the concurrent activity patterns of cell HN(L,2) and cell HE(L,3) while the left side underwent a transition from the peristaltic to the non-peristaltic coordination mode. As can be seen, the relative phase of the activity cycles of cell HN(L,2) (as judged by its impulse bursts) and of cell HN(L,3) (as judged by its associated large-amplitude IPSP’s
in cell HE(L,3)) remained constant while the phase of the activity cycle of cell HN(L,X) (as judged by its associated small-amplitude IPSP's in cell HE(L,3)) changed over the course of some six heartbeat cycles. These data and other similar data not shown here thus support the previous designation of cells HN(1), HN(2), HN(3) and HN(4) as fixed-phase interneurons.

The records of Figure 15 show the concurrent activity pattern of cells HE(R,3) and HN(R,7) while the right side underwent a transition from peristaltic to non-peristaltic coordination mode. This transition can be seen to be associated with an early burst of IPSP's in cell HE(R,3) due to cell HN(R,X), and a concurrent early burst of IPSP's in cell HN(R,7) due to one of the two HN(5) cells. Since the IPSP's of that early burst in cell HN(R,7) are greater in amplitude than the IPSP's recorded prior to the transition and known to be due to cell HN(L,5), the early burst must be due to inhibitory input from the previously inactive cell HN(R,5). Following the early IPSP bursts in cells HE(R,3) and HN(R,7), subsequent IPSP bursts again recur in regular sequence, one per heartbeat period, with the smaller amplitude IPSP's previously seen in cell HN(R,7) having ceased. The transition in coordination mode is now complete. The activity cycle of cell HN(R,X) has changed its phase relative to that of cell HN(R,3) (as judged by the occurrence of their respective IPSP's in cell HE(R,3)). And the activity cycle of cell HN(R,7) has changed its phase relative to that of HN(R,3) (as judged by the impulse bursts of cell HN(R,7) and the IPSP's due to cell HN(R,3) seen in cell HE(R,3)), because the previously inactive cell HN(R,5) became active and the previously active cell HN(L,5) became inactive. In records not presented here, a similar transition was observed.
Fig. 15. A coordination mode transition from peristaltic to non-peristaltic in a variable-phase cell HN(7) and ipsilateral cell HE(3). The two sets of traces shown form a continuous record. The transition began at the first arrow and ended at the second.

Fig. 16. Alteration of the coordination mode of an ipsilateral HE cell by intracellular injection of current into cell HN(L,5). The three sets of three traces shown form a continuous record. At the beginning of the record HE(L,4) was coordinated non-peristaltically and cell HN(L,5) was phasically active. When cell HN(L,5) was then rendered inactive with hyperpolarizing intracellular current injection, HE(L,4) became coordinated peristaltically. When HN(L,5) then resumed phasic activity upon cessation of current injection HE(L,4) again became coordinated non-peristaltically. (When cell HN(L,5) was hyperpolarized, no antidromic spikes invaded its cell body, indicating that it has a single impulse initiation site located in ganglion five). CM: Current injected into cell HN(L,5)
in cell HN(6) and the inverse transition from the non-peristaltic to the peristaltic coordination mode has been observed in both cell HN(6) and cell HN(7).

These data thus support the previous designation of cells HN(6) and HN(7) as variable-phase interneurons and the conclusion that the change in the phase of their activity cycles relative to the fixed-phase interneurons HN(1), HN(2), HN(3) and HN(4) during the transition in coordination mode is attributable to the conversion of one HN(5) cell from inactivity to activity and of the other HN(5) cell from activity to inactivity.

**Induced Transitions of the Coordination Mode**

No spontaneous transition in heartbeat coordination mode has yet been observed while an intracellular record was being taken from the critical cell HN(5), probably microelectrode penetration depolarizes the cell sufficiently to maintain it in an active state. However, under these conditions transitions in coordination mode can be induced by passage of current into cell HN(5), as shown in the record of Figure 16. At the outset of this record, the left side was coordinated in the non-peristaltic mode (as judged by the IPSP pattern in the record of cell HE(L,4) and cell HN(L,5) was phasically active, in accord with the schema of Figure 9B. Upon passage of hyperpolarizing current into cell HN(L,5) and cessation of its impulse activity, an immediate transition of cell HE(L,4) from the non-peristaltic to the peristaltic coordination mode is manifest. Upon release of cell HN(L,5) from hyperpolarization by current passage and resumption of its impulse burst activity, cell HE(L,4) immediately reverts to the non-peristaltic...
tic coordination mode. Similar induced transitions in coordination mode have been observed in four different preparations.

The records of Figure 17 present an experiment similar to that of Figure 19, except that here an HE motor neuron was monitored contralateral to the HN(5) cell into which a microelectrode had been inserted. At the beginning of this record hyperpolarizing current was being passed into cell HN(L,5) and, as would be expected, cell HE(R,4) on the side opposite to the inactive cell HN(L,5) received its IPSP input in the non-peristaltic mode. Upon release of HN(L,5) from hyperpolarization and resumption of its impulse burst rhythm, the IPSP input pattern to the contralateral cell HE(R,4) immediately changed in accord with expectation, to the peristaltic coordination mode.

Although in the experiment presented in Figure 17 manipulation of the HN(5) cell did result in an induced transition of coordination mode of the contralateral HE cell, this result is exceptional in that in most such experiments carried out in this study, passage of current into an HN(5) cell altered the heartbeat coordination mode only in the ipsilateral but not in the contralateral heart control neurons. Under these experimental conditions both sides were thus induced to take on the same—peristaltic or non-peristaltic—coordination mode, a state which is most unusual under normal conditions. In view of
the predominantly ipsilateral effect on the coordination mode of the manipulation of cell HN(5), it seems likely that there is no direct effect of the activity or inactivity of one HN(5) cell on the activity or inactivity of its contralateral homolog. This conclusion is supported by experiments such as that presented in Figure 16, in which the effect of current passage into one HN(5) cell on the other was monitored by a simultaneous recording from cell HN(7). At the start of the record of Figure 18A cell HN(R,5) was active and its impulses accounted for all the IPSP's observed in cell HN(R,7). Accordingly, the contralateral cell HN(L,5) was inactive, with the right side in the non-peristaltic and the left side in the peristaltic coordination mode. In the middle of the record of Figure 18A, when cell HN(R,5) was rendered inactive by passage of hyperpolarizing current into it, all IPSP input to HN(R,7) ceased and the phase of the activity cycle of cell HN(R,7) was now maintained by its EPSP input from the ipsilateral cells HN(3) and HN(4). Hence, here cell HN(L,5) remained inactive despite the induced inactivity of cell HN(R,5) and both HN(5) cells remained inactive as long as hyperpolarizing current was passed into cell HN(R,5). At a later time in this experiment there occurred a spontaneous transition in coordination mode, so that the previously inactive cell HN(L,5) became active. Now, as shown in Figure 18B, bursts of IPSP's continued to appear in cell HN(R,7) during passage of hyperpolarizing current into cell HN(R,5). In the middle of the record of Figure 18B, when cell HN(R,5) was released from hyperpolarization, two antiphase sets of IPSP's appear in cell HN(R,7), indicating that both HN(5)'s are active, albeit antiphasically. Here both HN(5) cells remained active as long as cell HN(R,5) was maintained in an active state.

Control of the Coordination Mode by Cell HN(5)

Cell HN(5) controls the coordination mode of the heart motor neurons in two different ways. First, as far as HE cells posterior to ganglion 6 are concerned, the active member of the HN(5) cell pair determines their coordination mode by causing the activity cycles of both right and left members of the HN(5) and HN(7) cell pairs to take on approximately the same phase as the cycles of the HN(3) and HN(4) cells ipsilateral to the active cell HN(5) and therefore about 180° out of phase with the cycles of the HN(3) and HN(4) cells contralateral to the active cell HN(5). Thus on the side of the active cell HN(5), all interneurons providing inhibitory input to the motor neurons posterior to ganglion 6 are active in about the same phase, and hence that side is necessarily coordinated in the non-peristaltic mode. On the opposite side, however, that of the inactive cell HN(5), these interneurons are active in a phase progression, and hence that side is coordinated in the peristaltic mode.

Second, as far as the HE cells anterior to ganglion 7 are concerned, the active member of the HN(5) cell pair appears to determine their coordination mode by causing the activity cycle of its ipsilateral cell HN(X) to be approximately 180° out of phase with the cycles of its ipsilateral cells HN(3) and HN(4), while allowing the activity cycle of its contralateral cells HN(X), HN(3)
Fig. 19 A and B. Correlation of action potentials in cell HN(5) with HN(X) IPSP's in ipsilateral anterior HE cells. Both panels show superimposed oscilloscope sweeps triggered by action potentials in cell HN(5) followed at a constant delay by an IPSP in an ipsilateral HE cell. Eleven and ten superimposed sweeps in Panels A and B respectively.

and HN(4) to be approximately in phase with each other. Thus on the side of the active cell HN(5), HN(X) and the other interneurons providing inhibitory input to the motor neurons anterior to ganglion 7 are active in antiphase, thus producing the two separate bursts of small- and large-amplitude IPSP’s characteristic for the frontmost HE cells during coordination in the non-peristaltic mode. On the opposite side, however, that of the inactive cell HN(5), cells HN(X), HN(3) and HN(4) are active nearly in phase, thus producing the intermeshed bursts of small- and large-amplitude bursts of IPSP’s characteristic for the frontmost HE cells during coordination in the peristaltic mode.

To understand how cell HN(5) can determine the phase of the activity cycle of the HN(X) cell pair it is necessary to consider that in the peristaltic mode, the impulses of cell HN(X) arise in a ganglion anterior to ganglion 4 and travel rearward in its intersegmental axon, whereas in the non-peristaltic mode its impulses arise in a ganglion posterior to ganglion 5 and travel forwardward (Thompson and Stent, 1976c). Hence, in its governance of the activity of the HN(X) cell pair, cell HN(5) must be able to control an anterior impulse initiation site, to be referred to as HN(X)A, and a posterior impulse initiation site, to be referred to as HN(X)P (cf. Fig. 9 B). A most important clue to the mechanism of control of HN(X) cell activity is provided by the finding presented in Figure 19A, where it can be seen that individual impulses recorded from cell HN(L,5) are followed in cell HE(L,4) with a constant delay of about 200 ms.
by low-amplitude IPSP's attributable to cell HN(L,X) impulses. Since during the taking of this record the left side was coordinated in the non-peristaltic mode, these impulses must have arisen at the posterior, or HN(L,X)_p initiation site. Hence, it can be concluded that there is a strong excitatory synaptic or electrical link between cell HN(5) and the ipsilateral HN(X)_p initiation site, so that a single HN(5) impulse can give rise to an HN(X)_p impulse.

This conclusion is supported by the, at first sight, surprisingly long delay of about 200 ms between the initiation of an impulse in cell HN(5) in ganglion 5 (where, as previously inferred (Thompson and Stent, 1976c) and confirmed in this study (cf. Fig. 16) the only impulse initiation site of cell HN(5) is located) and the appearance of a matching IPSP in cell HE(4) one ganglion to the front. This delay appears surprisingly long because the intergangionic travel time of HN cell impulses is only about 40 ms per segment (Thompson and Stent, 1976b and Table I). Moreover, as seen in the records of Figure 19B, individual HN(5) impulses are followed by an IPSP in the ipsilateral cell HE(6) one ganglion to the rear with a constant delay of only about 50 ms. But since the HN(X)_p initiation site lies in a ganglion posterior to ganglion 5, then each HN(5) impulse must travel rearward for at least one segment to ganglion 6, cause there the initiation of an impulse at HN(X)_p; this HN(X)_p impulse must then travel frontward for at least two segments to ganglion 4 to produce the HE(4) cell IPSP with which the HN(5) impulse is matched. Thus, in view of this circuitous route, it is to be expected that the HE(4) IPSP's follow HN(5) impulses with a much longer delay than do the HE(6) IPSP's.

The postulated strong excitatory connection between HN(5) and HN(X)_p therefore explains how in the non-peristaltic side, bursts of forward-traveling HN(X) impulses arise at the posterior HN(X)_p initiation zone, in the same phase as the impulse bursts of the ipsilateral cell HN(5) and hence about 180° out of phase with the impulse bursts of the ipsilateral cells HN(3) and HN(4): the HN(X) impulse bursts are driven, impulse for impulse, by HN(5) impulses. Conversely, on the peristaltic side, no impulse bursts arise at the HN(X)_p site at all, because here the ipsilateral HN(5) is inactive. To account for the fact that on that peristaltic side rearward-traveling HN(X) impulses arise at the anterior initiation site HN(X)_a, it can be assumed, as was done previously (Thompson and Stent, 1976b) that the posterior initiation site is "dominant" over the anterior site: HN(X)_a is active in impulse initiation if and only if HN(X)_p is inactive. In support of this proposal it can be seen in the record of Figure 16 that upon hyperpolarization of HN(L,5) by current passage, the previously active HN(L,X)_p site became inactive and the previously inactive HN(L,X)_a site became active during the next heartbeat cycle. (It is to be noted, however, that in other, similar experiments the inactive HN(X)_a site became active only after two or more heartbeat cycles had elapsed following the induced cessation of activity of the HN(X)_p site by hyperpolarization of the ipsilateral cell HN(5).) Moreover, to account for the fact that the phase of the impulse bursts now initiated at the HN(X)_a site was shifted by 180° relative to the phase of the impulse bursts previously initiated at the HN(X)_p site (and hence in about the same phase as the impulse bursts of the ipsilateral cells HN(3) and HN(4), it is assumed that the HN(X)_a site receives some un-
identified phasing synaptic input from one or more other HN cells (for instance, inhibitory input from the ipsilateral cells HN(1) and HN(2) as shown in Fig. 9B) which causes the HN(X) activity cycle to be in phase with that of the ipsilateral cells HN(3) and HN(4).

Hence, due to its strong excitatory influence on the "dominant" posterior initiation site, cell HN(5) can control at which of the two potential initiation sites—HN(X)p or HN(X)p—and in which phase, in phase or out of phase with the ipsilateral cells HN(3) and HN(4)—the HN(X) impulse bursts are initiated.

Control of Activity or Inactivity of Cell HN(5)

The determination of the heartbeat coordination mode is thus focused on the reciprocal transition of the HN(5) cell pair from phasic activity to complete inactivity. To explain how this transition is effected, it is useful to assume that the single impulse initiation site of cell HN(5) in ganglion 5, unlike the multiple, segmentally iterated impulse initiation sites of the other identified heart interneurons, does not possess an endogenous activity rhythm. Instead, one may envisage that the impulse burst rhythm of cell HN(5) is driven by rhythmic excitatory input to its impulse initiation site. Such input would be provided by the bursts of EPSP's which, as the record of Figure 11A revealed, the active HN(5) cell on the non-peristaltic side receives concurrently with its impulse burst and 180° out of phase with the IPSP bursts which cell HN(5) receives from the ipsilateral HN(3) and HN(4) cells. Accordingly, the contralateral HN(5) cell on the peristaltic side would then be inactive either because it does not receive any EPSP input at all or because the EPSP input which it does receive is cancelled by occurring in the same phase as the IPSP input from the ipsilateral HN(3) and HN(4) cells.

At least two crucial points remain to be elucidated under this proposed explanation of the control of the activity of cell HN(5). The first of these is the source of the rhythmic EPSP input, about which nothing is known other than, as shown by the experiment of Figure 11B, the EPSP's appear to be due to impulses traveling in an ipsilateral intersegmental axon extending at least from ganglion 3 to ganglion 7.

The second of these points, probably related to the first, concerns the right-left coordination of the transition of cell HN(5) from inactivity to activity. As was seen in the preceding, spontaneous transitions in heartbeat coordination mode are invariably reciprocal, thus coupling in some manner the transition of one HN(5) cell from activity to inactivity with the reverse transition of the other HN(5) cell from inactivity to activity. However, as was also seen in the preceding, this coupling cannot be a direct one between the HN(5) cell pair itself, since by passing current into an HN(5) cell it is possible to induce hemilateral transitions in coordination mode, under which the HN(5) homologs are either both active or both inactive. No information is as yet available concerning the evidently indirect pathway by which the inactive state of one HN(5) is linked to the active state of its contralateral homolog.
Alternate Heartbeat Coordination States in the Leech

Fig. 20. The effects on the coordination modes of an anterior bilaterally HE cell pair, of the removal of ganglion six in a preparation consisting of the head brain through ganglion six. Prior to cutting the 5-6 interganglionic connectives cell HE(R,3) was coordinated non-peristaltically and cell HE(L,3) was coordinated peristaltically. After the cut indicated by the arrow, both cells were coordinated peristaltically. The bottom two traces are a time expansion of the indicated parts of the top two traces, that allow closer inspection of the IPSP's patterns in the two HE(3) cells.

Effect of Ganglionic Ablations on the Coordination Mode

To further support the schematic HN cell circuit diagram of Figure 12B, the effects on the heartbeat coordination of a series of ganglionic ablations of the nerve cord were examined. In one such ablation experiment, records were taken from the HE(3) cell pair in a preparation consisting of the head brain through ganglion 11. Posterior ganglia were removed one at a time by cutting interganglionic connectives and the effects on the coordination mode of HE cells were observed. The serial removal of ganglia 10, 9, 8 and 7 each had no discernible effect on the coordination mode of the anterior HE cells. Removal of ganglion 6, however, had a dramatic and permanent effect on the coordination mode, as is shown in Figure 20. As judged by the phasing of the bursts of small-amplitude IPSP's (due to cell HN(X)) and large-amplitude IPSP's (due to cell HN(3)), prior to removal of the sixth ganglion the right side was coordinated in the non-peristaltic and the left side in the peristaltic mode, hence both HN(X) and HN(X) impulse initiation sites were intact. However, after the ablation, both sides were coordinated in the peristaltic mode, as indicated by the in-phase occurrence of IPSP's due to HN(X) and HN(3). These findings show that the posterior, or HN(3) initiation site which is active in the non-peristaltic coordination mode is located in ganglion 6 (as indicated in Figure 9B), because it is only after removal of this ganglion that HN(X) activity ceases. Moreover, these results show that the inactivity (or in this case, the absence) of the "dominant" HN(X) site is a necessary and sufficient condition for
Fig. 21 A and B. Coordination modes of bilateral HE cell pairs in preparations missing anterior ganglia. Panel A. The two sets of traces shown form a continuous record taken from a preparation consisting of ganglia 5 through 11. A reciprocal coordination mode transition occurred at the arrow in the HE cell pair. Side to side coordination was impaired, but always the HE cell on one side was coordinated peristaltically while its contralateral homolog was coordinated non-peristaltically. Panel B. The records were taken from a preparation consisting of ganglia 6 through 10. No HN(X) IPSP's were observed in either member of the bilateral HE cell pair and side to side coordination was completely lost.

the activity of the HN(X), initiation site and the appearance of the peristaltic coordination mode. It is evident, furthermore, that after the ablation, the peristaltically controlled activity cycle of each of the two contralateral HN(X), initiation sites occur approximately 180° out of phase with one another and in phase with the fixed-phase activity cycle of their ipsilateral HN(3) cell. This suggests that the two contralateral HN(X), initiation sites receive their previously inferred phasing synaptic input from antiphase, fixed-phase contralateral HN cell homologs, in ganglia anterior to ganglion 6, such as the HN(1) and HN(2) cell pairs (as shown in Figure 9B).

Removal of ganglia anterior to ganglion 4 does not interfere seriously with the normal bilateral coordination of the peristaltic and non-peristaltic heartbeat modes in the posterior ganglia of the cord. For example, the records of Figure 13A showing a normal, spontaneous transition of the coordination mode in the HE(4) cell pair were actually taken from a nerve cord preparation which
consisted only of ganglia 4 through 11. This finding indicates that the axons of cells HN(1), HN(2), HN(3) and HN(X) not only possess impulse initiation sites with endogenous impulse burst rhythms in ganglia 4 but also that these initiation sites in ganglion 4 receive the necessary synaptic inputs to ensure that their activity cycles are locked into an appropriate phase relation.

The records of Figure 21A show the coordination mode of posterior HE cells after the ablation of ganglion 4. As can be seen, at the beginning of this record, cell HE(R,5) was coordinated in the peristaltic and cell HE(L,5) in the non-peristaltic mode. In the middle of this record a spontaneous transition in coordination mode occurred after which cell HE(R,5) was coordinated in the non-peristaltic and cell HE(L,5) in the peristaltic mode. However, it is evident that despite the reciprocal nature of the right-left transition in coordination mode, the heartbeat rhythm of the two sides of this preparation was not coordinated in its normal manner: the heartbeat period is irregular and unequal on the two sides and the HE(R,5), and HE(L,5) activity cycles bear no fixed phase relation to each other. To account for the finding that both peristaltic and non-peristaltic coordination modes can be maintained and undergo reciprocal right-left transitions in the absence of ganglion 4 it is necessary to envisage that the axons of cells HN(1), HN(2), HN(3), HN(4) and HN(X) also possess impulse initiation sites with endogenous impulse burst rhythms and some appropriately phased synaptic inputs also in ganglion 5 and that the elements of the unaccounted for indirect pathway by which the inactive state on one HN(5) cell is linked to the active state of its contralateral homolog are present in ganglia posterior to ganglion 4. The failure to maintain the normal right-left coordination of the heartbeat rhythm in the absence of ganglion 4 must mean, however, that the impulse initiation sites of cells HN(3) and HN(4) in ganglion 5 lack the reciprocal inhibitory connections to their contralateral homologs which, according to the circuit diagram of Figure 9A are known to be present in the ganglia in which their cell bodies are located. Thus, whereas in this truncated preparation the proper phase relation between the ipsilateral ensemble of fixed-phase HN cells is still maintained, ablation of ganglion 4 evidently removed the last right-left phase coupling and caused the oscillatory interneurons of the two sides to become free-running with respect to each other.

Finally, the records of Figure 21B show that after ablation of ganglion 5 the pair of HE(6) cells of the truncated nerve cord have lost their IPSP input from the HN(X) cell pair, and hence cannot be diagnosed with respect to their peristaltic or non-peristaltic coordination mode. The disappearance of HN(X) IPSP's from cell HE(6) supports the previous contention that the impulse burst rhythm of the posterior HN(X) initiation site, HN(X)p, which is located in ganglion 6, is generated by the impulse burst rhythm of the ipsilateral cell HN(5), since upon removal of ganglion 5 and disconnection from the unique HN(5) impulse initiation site, both right and left HN(X)p sites would be expected to become inactive.

*Is Cell HN(X) a Part of Cell HN(5)?*

The finding of the experiment of Figure 19 that there is a close correspondence between impulses recorded from cell HN(5) and impulses initiated at the poste-
Fig. 22. Schematic representation of the minimal necessary anatomy of cell HN(5) consistent with it being cell HN(X). Three impulse initiation sites are labeled HN(X)p and one is labeled HN(X)q. According to this model, the HN(X)p, initiation site in ganglion 3 is “dominant” over the other HN(X)p sites and the HN(X)q site is “dominant” over all. The HN(X)p sites can all produce impulse bursts endogenously, but only the “dominant” one is normally expressed. However, they are all “suppressed” when the non-endogenous HN(X)q site is driven to activity by excitatory synaptic input. The branch point labeled “somatofugal impulse conduction only” allows impulses to invade the anterior going branch from the main axon but not vice versa.

rior initiation site of the unidentified cell HN(X) raises the possibility that HN(5) is HN(X) and that HN(X)p is located in a process of the ipsilateral cell HN(5). However, if this were the case it would be necessary to postulate a rather complicated structure of the HN(5) axon. That axon, which is known to project rearward at least as far as ganglion 7, would have to form a branch in ganglion 6 that projects forward to ganglion 3, making inhibitory synaptic contacts with the ipsilateral cells HE(6), HE(5), HE(4) and HE(3) as it transits their ganglia, as shown schematically in Figure 22. This forward branch would represent the HN(X) axon and contain the HN(X)p set of anterior impulse initiation sites. The HN(X)q, or posterior initiation site lacking an endogenous rhythm could then be identical with the previously identified impulse initiation site of cell HN(5) in ganglion 5, with the earlier (and in this case, false) assignment of the HN(X)p site to ganglion 6 being due to the forward looping branch of the HN(5) axon in ganglion 6. However, in addition to postulating the forward looping branch in ganglion 6, it would be necessary to attribute also special electrical properties to the branch point in order to maintain that cell HN(5) is cell HN(X). These special electrical properties would have to provide for impulse transmission across the branch point with a high safety factor in the somatofugal and with a very low safety factor in the somatopetal direction (with respect to the HN(5) soma). The postulation of somatopetal branch point transmission failure is necessary to account for the fact that on the peristaltic side the HN(X)p initiation zone is rhythmically active while the main rearward-going HN(5) axon, on whose side branch HN(X)p would be situated, is evidently inactive. (That is to say the inactive HN(5) cell on the
peristaltic side shows no antidromic impulses in its cell body and causes no IPSP's in the HN(6) and HN(7) cell pairs.) Moreover, as the experiment presented in Figure 11 B showed, direct electrical stimulation of the 3–4 interganglionic connective, where the forward-going HN(5) axon branch ought to be present, does not evoke antidromic impulses in the HN(5) cell body, whereas similar stimulation of the 6–7 interganglionic connective where the main rearward-going HN(5) axon is present, does evoke such impulses. Indeed, it is necessary to postulate also the possibility of occasional somatofugal branch point transmission failure, since detailed analysis of the records from which the composite data of Figure 19 were generated showed that on the non-peristaltic side only most, but not all, impulses recorded from the HN(5) cell body are matched with constant delay by an IPSP in an anterior HE cell attributable to an impulse in the HN(X) axon. Thus on the basis of the data available at present it is difficult to decide whether cells HN(X) and HN(5) are two different, strongly coupled cells, or whether the HN(X) axon is a frontward projecting branch of the HN(5) axon originating in ganglion 6 and connected to the main axon via a branch point that permits only somatofugal impulse transmission.

Discussion

Regional Differentiation among Heart Interneurons

As is implied in Figure 9 the identified HN cell population can be subdivided into four regional subgroups that have different electrical, synaptic, and functional properties: 1) Cells HN(3) and HN(4)). 2) Cells HN(1) and HN(2). 3) Cells HN(6) and HN(7). 4) Cell HN(5).

Ipsilateral cells HN(3) and HN(4) are active in phase with each other, and in antiphase with their contralateral homologs. Reciprocal inhibitory synaptic connections between bilateral homologs insure antiphase activity and the electrical coupling of ipsilateral serial homologs insures in phase activity. Both the HN(3) and HN(4) cell pairs provide phasing inhibitory synaptic input to ipsilateral HE cells and ipsilateral cell HN(5). Therefore, the function of the HN(3) and HN(4) cell pairs is to generate and coordinate the HN cell rhythm and to phase ipsilateral HE cells.

Ipsilateral cells HN(1) and HN(2) are also active in phase with each other, and in antiphase with their contralateral homologs. The antiphase activity of the bilateral homologs indicates reciprocal inhibitory synaptic interconnections, while the in phase activity of ipsilateral serial homologs indicates that they are electrically coupled. Cells HN(1) and HN(2) provide phasing inhibitory synaptic input to other HN cells (ipsilateral cells HN(3) and HN(4) and presumably the ipsilateral HN(X) impulse initiation site). Unlike other HN cells they do not provide inhibitory synaptic input to HE cells and they have no impulse initiation site in their ganglion of origin. Therefore, the function of the HN(1) and HN(2) cell pairs is to generate and coordinate the HN cell rhythm.
All the cells of the HN(6)–HN(7) cell group are active in phase. They have no synaptic connections among themselves; their in phase activity results from the phasing inhibitory synaptic input they all receive from the single active HN(5) cell. Both the HN(6) and HN(7) cell pairs provide phasing inhibitory synaptic input to ipsilateral HE cells but not to any identified HN cells. Therefore, the function of the HN(6) and HN(7) cell pairs is to generate but not to coordinate the HN cell rhythm and to phase ipsilateral HE cells.

The HN(5) cell pair has a number of unique properties among HN cells. Unlike the other identified HN cells a cell HN(5) appears to require excitatory synaptic drive to be active (cf. Figure 11) and therefore not to produce impulse bursts endogenously. Additionally it has only a single impulse initiation site, located in its ganglion of origin. Cell HN(5)'s synaptic interactions with other HN cells are also unique; it provides bilateral inhibitory synaptic input to the HN(6) and HN(7) cell pairs. Like cells HN(6) and HN(7), cell HN(5) has no synaptic connections with its contralateral homolog and is phased by inhibitory synaptic input from more anterior HN cells. Cell HN(5) additionally controls, presumably by excitatory output, the ipsilateral HN(X), impulse initiation site. The function of cell HN(5) is to coordinate the HN cell rhythm. Also cell HN(5) is of prime importance in the establishment of the two alternate heartbeat coordination modes, to be discussed below.

Hence, even though the HN cells represent a group of serial homologs, they show a remarkable degree of differentiation among themselves, both in their synaptic connections with HE cells and other HN cells and in their electrical characteristics. This regional differentiation in HN cell properties results in the functional differences among them.

**Cell HN(5) and the Control of Peristalsis and Non-Peristalsis in the Leech Heartbeat System**

In the leech heartbeat system two alternate coordination states exist, right side peristaltic, left side non-peristaltic or vice versa. These two states result from differences in the phasing between anterior (fixed-phase) and posterior (variable-phase) HN cells on the two sides. This difference in phasing on the two sides is mediated by the HN(5) cell pair of which only one member is phasically active at a time, for 10–50 heartbeat cycles. Cell HN(5) interfaces the fixed-phase and variable-phase HN cells by receiving inhibition ipsilaterally from fixed-phase cells HN(3) and HN(4) and providing inhibition bilaterally to the variable-phase cells HN(6) and HN(7). On the side of the phasically active cell HN(5) the fixed-phase cells HN(3) and HN(4) are active in phase with the variable phase cells HN(6) and HN(7) and (cf. Fig. 12B) the side is therefore coordinated non-peristaltically (Fig. 13). On the side of the completely inactive cell HN(5) the fixed phase cells HN(3) and HN(4) are active in antiphase with the variable phase cells HN(6) and HN(7) and (cf. Fig. 12B) the side is therefore coordinated peristaltically (cf. Fig. 13). It appears that cell HN(5) does not produce impulse bursts endogenously (at least on its main axon, cf. Figure 22) but must be driven to activity by excitatory input by an as yet unidentified pathway (cf.
Figure 11). This pathway, by determining which member of the HN(5) cell pair will be active, determines the phasing between fixed and variable phase cells, and hence the coordination modes of the two sides.

Cells similar to the HN(5) cell pair may interface endogenously active cells or oscillatory networks on other systems and may be responsible for the variable phasing of particular components of the system associated with different behavioral states. Such differences exist, e.g., in the phasing of elevator and depressor versus protractor and retractor motor neurons associated with forward versus rearward walking in lobsters (Ayers and Davis, 1977).

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