SUMMARY AND CONCLUSIONS

1. The interactions among the four pairs of interneurons (HN(1)–HN(4)) of the heartbeat timing oscillator are confined to the third and fourth ganglia (G3 and G4). In isolation, G3 and G4 each produces a rhythm essentially the same as that shown when the two ganglia are linked together.

2. The local circuits in both ganglia have the same general form. In both the oscillation centers on a bilateral pair of HN cells that are linked by reciprocal inhibition (the HN(3) pair in G3 and the HN(4) pair in G4). In addition, there is reciprocal inhibition between an HN(3) or HN(4) cell and the intersegmental processes of the ipsilateral HN(1) and HN(2) cells.

3. These connections account for the phase relationships in an isolated G3 or G4, since cells linked by reciprocal inhibition produce bursts in alternation.

4. In isolated ganglia, reciprocal inhibition not only coordinates the activity of the HN cells but also appears to help generate their bursts.

5. Yet reciprocal inhibition alone cannot account for the activity of the network. An endogenous property of the HN(3) and HN(4) cells appears to create the instability necessary for oscillation.

INTRODUCTION

A network including seven pairs of interneurons (HN cells) controls the motor neurons that drive the rhythmic constrictions of the lateral heart tubes of the medicinal leech (5, 28). HN somata lie on the left and right sides of the seven most rostral segmental ganglia (G1–G7) and are indexed accordingly: HN(L, 1) HN(R, 1), . . . , HN(L, 7), HN(R, 7). (The designation of side is often dropped when making generalizations.) The HN(1)–HN(4) pairs constitute the heartbeat “timing oscillator” by virtue of their capacity to reset the phase of the entire HN network (22). All measurable properties of the timing oscillation in G1–G4 persist when that portion of the nerve cord is isolated (3, 22, 30–32, and the present report).

Each HN cell sends an axon caudally to more posterior ganglia but sends no processes rostrally (25, 31). The HN(2) and HN(3) cells extend at least to G4 and the HN(1) cell extends beyond G3 (and therefore presumably to G4) (13; unpublished results), supporting inferences made from physiological results (3, 32). Thus, within the G1–G4 chain each ganglion contains a pair of HN somata plus processes of the HN cells that originate in more rostral ganglia. This spatial distribution allows one to decompose the timing oscillator into four simpler units by severing the intersegmental connectives.

It is known from previous work that the HN(1) and HN(2) processes within G1 and G2 play no role in generating oscillation. Neither cell initiates spikes in G1 or G2 and neither cell receives detectable synaptic input in its own ganglion (3, 32). Moreover, when the G2-G3 connective is severed, the HN(1) and HN(2) cells produce only damage discharge on penetration, whereas the activity of the network caudal to G2 (including that of the severed axons of the HN(1) and HN(2) cells) is unaltered. Finally, compared
to other HN cells, the HN(1) and HN(2) cells have reduced dendrites in G1 and G2, respectively, reflecting their reduced function (25).

The essential components of the timing oscillator are therefore confined to G3 and G4, both of which oscillate autonomously when isolated from the rest of the nerve cord. The present paper shows that the local synaptic connections among the HN(1)-HN(4) pairs account for the residual oscillation produced by G3 or G4 in isolation if one attributes a plausible endogenous property to the HN(3) and HN(4) cells. The following paper (21) shows how the circuits in the two ganglia are coordinated to produce the output that drives the other neurons of the heartbeat network.

METHODS

Leeches (Hirudo medicinalis) were obtained from a commercial supplier (Ricarimpex). Experiments were conducted on isolated segmental ganglia (singly or in chains) maintained in leech physiological saline (17) at room temperature. Current was injected intracellularly through the recording electrode, using a bridge circuit. With the following exception, all procedures are described elsewhere (6, 30).

Lucifer yellow-induced photoinactivation

The method of Miller and Selverston (14) was used to inactivate segments of neurons. Lucifer yellow (29) was injected, iontophoretically (5-10 nA) or with pressure (5-30 psi), sufficient to turn the soma distinctly yellow in white light. Ganglia were then irradiated for 5 min with intense blue light in an apparatus similar to that described by Selverston and Miller (24) and washed 30 min in saline. Experiments were completed within the next 30 min.

Since the photoinactivation procedure had not been used previously in the leech central nervous system (CNS), it was tested on the well-studied S-cell system (7, 9, 16) prior to use in critical experiments. The single S-cell in each ganglion sends an axon rostrally and caudally, forming an electrical junction in the connective with the two adjacent S-cells. Together the S-cells form a linear network in which an impulse initiated anywhere propagates in both directions to all cells in the series (Fig. 1A). Lucifer yellow injection itself caused no damage (Fig. 1B), but subsequent irradiation with blue light abolished the resting potential and blocked initiation and conduction (Fig. 1C, D). Uninjected neurons including cell 204 (35), which lay adjacent to the S-cell, were undamaged.

RESULTS

Monitoring inactivation of HN processes

The impulses of an HN(3) cell normally arise in G3 and propagate to G4, producing large inhibitory postsynaptic potentials (IPSPs) in the ipsilateral heart motor neurons, HE(3) and HE(4) (31), (Fig. 2). If initiation in G3 is blocked, the impulses arise at a secondary initiation site in G4 and propagate antidromically to G3 (6, 31). Photo-inactivation of the G3 processes of an HN(3) cell was taken to be successful and selective if an HN(3) impulse initiated in G4 produced no IPSP in the ipsilateral HE(3) cell (Fig. 2B) while IPSPs from another input, HN(X) (3, 31), persisted. Similar methods were used to monitor the inactivation of the G4 processes of treated HN(4) cells.

HN cell processes in G3 form a local oscillator

Since there appear to be no connections in G1 or G2 between the HN cells, one can use the chain G1-G3 to study synaptic interactions in G3. In such preparations the two HN(3) cells burst in alternation (Fig. 3A), a phase relationship maintained by reciprocal inhibition. Several criteria, including one-to-one matching of impulses and IPSPs (Fig. 3B, C), indicate that the connection is monosynaptic (18, 32).

An HN(1) cell is also phasically active in G3, producing bursts that alternate with those of the ipsilateral HN(3) cell (Fig. 4A). Spontaneous impulses in the soma of an HN(1) cell lag behind matching IPSPs in the ipsilateral HN(3) cell, whereas impulses induced by current passed into the soma lead IPSPs by a similar margin (Fig. 4A), indicating that spontaneous impulses arise in G3 and propagate antidromically to G1 (3). The HN(1)-induced IPSPs are small and often difficult to detect against the background of impulses and PSPs in the HN(3) cell, but they can be seen clearly when the contralateral HN(3) cell is inactivated (Fig. 4B). The HN(2) cell initiates impulses in G3 that produce IPSPs in the ipsilateral HN(3) cell followed by spikes in the HN(2) soma.
FIG. 1. Test of the photoinactivation procedure. The preparation was a chain of ganglia G8–G13. The S impulse was monitored in the G7–G8 and G13–G14 connectives. In each section the diagram shows the placement of the intracellular electrode in one S-cell. Top trace: extracellular recording from G7–G8 connective. Middle trace: extracellular recording from G13–G14 connective. Bottom trace: intracellular recording from the S-cell indicated in the schematic drawing. Voltage bar, 70 mV in A and B, 50 mV in C and D. Six superimposed sweeps triggered on spontaneous intracellular spike are shown in each case. A: before dye injection. B: after pressure injection of lucifer yellow dye into S(11) but before irradiation with blue light. C: after irradiation. Impulses induced in S(10) failed to conduct through G11 to the right end of the chain. (Shading shows the irradiated section of the S(11) cell.) D: after irradiation. Impulses induced in S(12) failed to conduct through G11 to the left end of the chain.

(Fig. 4C) and, like the HN(1) cell, the HN(2) cell produces bursts from its G3 site that alternate with those of the ipsilateral HN(3) cell (22, 32). The HN(3) cell strongly inhibits the G3 initiation sites of the HN(1) and HN(2) cells (22) and, as Maranto (13) has recently shown, the inhibition from HN(3) to HN(2) is monosynaptic. Hence, each HN(3) cell is linked by reciprocal inhibition to both the contralateral HN(3) cell and the ipsilateral HN(1) and HN(2) cells.

Known connections in G3 provide a complete description of rhythmic pattern

In the minimal network (Fig. 5A) reciprocal inhibition locks an HN(3) cell in antiphase with both its contralateral homologue and the ipsilateral HN(1) and HN(2) cells. (Since ipsilateral HN(1) and HN(2) cells appear to have identical properties and function, I represent them as a single unit in all circuit diagrams.) If, as shown, the reciprocal connection between the HN(3) cells is the only link across the midline, severing that link should abolish correlation between activity in the left and right sides of the circuit. To test this prediction I inactivated one HN(3) cell both photodynamically and with hyperpolarizing current (in separate experiments). As a result, the activity of the HN(1) and HN(2) cells became uncorrelated with that of the contralateral HN(3) cell (Fig. 5) (the source of the large IPSPs seen in the hyperpolarized HN(3) cell). As discussed be-
FIG. 2. Monitoring the inactivation of HN cells. The preparation was G1–G4. A: synaptic inputs to the HE(3) and HE(4) cells. Solid lines denote cell processes, filled circles are inhibitory synapses, and the small square symbolizes the distal initiation site of the HN(3) cell. B: HE(3) and HE(4) cells received two classes of matching IPSPs. The larger IPSPs (dashed lines) were caused by the ipsilateral HN(3) cell (31); the smaller IPSPs (solid lines in C) were caused by the HN(X) unit (31). C: G3 processes of the ipsilateral HN(3) cell were photoinactivated in this preparation. Large IPSPs persisted in the HE(4) cell but not in the HE(3) cell; small IPSPs were present in both.

FIG. 3. Reciprocal inhibition in an isolated G3. A: paired intracellular recordings from the HN(3) cells. B, C: details of A revealed on a faster time base. Note that although most IPSPs were matched by impulses in the other HN(3) cell, there were some small unmatched IPSPs.
low, when an HN(3) cell is inactivated, the bursts of the contralateral HN(1)-HN(3) cells remain coordinated with one another (Fig. 6). On the basis of these two results one can deduce that the activity of HN(1) and HN(2) cells on opposite sides of the network becomes uncoordinated when one HN(3) cell is inactivated.

Thus the HN(1) and HN(2) cells have no functionally significant connections in G3 with any contralateral HN cell. Other observations support this conclusion: a burst induced in an HN(1) or HN(2) cell does not directly affect the activity of the contralateral HN cells in G3. In addition there appear to be no functionally significant connections between ipsilateral HN(1) and HN(2) cells, since a burst induced in an HN(1) cell fails to excite the ipsilateral HN(2) cell, and vice versa. Since the scheme of Fig. 5A is both necessary and sufficient to explain the observed interactions in G3, it appears to be complete.

**Reciprocal inhibition between HN(3) cells helps time oscillation in G3**

It was assumed (28) that the oscillation of the HN network was timed by endogenous polarization rhythms of the HN cells. Since the HN(1) and HN(2) cells were thought to be at the top of the synaptic hierarchy, it followed that the endogenous oscillation of these two pairs must be the ultimate source of timing in the network. In this scheme the endogenous polarization rhythms of the HN(1) and HN(2) cells produced oscillation, reciprocal inhibition maintained side-to-side coordination, and excitatory synapses coupled the HN(1) and HN(2) cells ipsilaterally. Given the additional synaptic connections identified recently (22) and the results presented in the preceding section, it is now unlikely that oscillation arises by the above mechanism. Neither the HN(1) nor HN(2) cell has a strong or reliable endogenous rhythm when phasic inhibition from the

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**FIG. 4. Physiology of the HN(1) and HN(2) cells.** The preparation was G1–G3. **A**: activity of the HN(1) and HN(3) cells. Small IPSPs in the HN(3) cell preceded spikes in the HN(1) soma. When the HN(1) cell was depolarized (CM, current monitor), the induced spikes led IPSPs. **B**: HN(1)-HN(3) synapse. The contralateral HN(3) cell was photoinactivated. The experiment was recorded on magnetic tape, then replayed in reverse. Six oscilloscope sweeps were triggered on the HN(1) spike; the result was photographed then reversed to restore the original time sequence. **C**: HN(2)-HN(3) synapse. Details as in **B**.
FIG. 5. HN(3) pair is essential for side-to-side coordination of G3. A: simplified schematic of the G1–G3 chain. In this and the following figures, schematics describe the experimental preparation. In these schematics, large circles are cells, lines are cell processes, small circles denote inhibition, and squares show impulse-initiation sites in more posterior ganglia. Dotted outlines show cells either photoinactivated prior to the experiment or hyperpolarized transiently during the experiment. B: HN(1) released from inhibition. When the HN(R, 3) cell was hyperpolarized, the HN(L, 1) cell burst weakly and irregularly with no temporal relation to the bursts of IPSPs in the HN(L, 3) cell and the frequency of the IPSP bursts decreased. C: HN(2) released from inhibition. The response of the HN(L, 2) cell on hyperpolarization of the HN(L, 3) cell was similar to that of the HN(1) cell in B.

HN(3) cell is removed (Fig. 5), and none of the connections invoked to explain coordination among the HN(1) and HN(2) cells appear to exist (Fig. 5A).

There are three other factors that are potentially important for timing: 1) the reciprocal connections between the HN(3) cells, 2) the reciprocal connections between cell HN(3) and the ipsilateral HN(1) and HN(2) cells, and 3) the endogenous properties of the HN(3) cells.

If reciprocal inhibition between the HN(3) cells did not contribute to oscillation but served only to coordinate activity in the two sides of the network, then breaking the link between the HN(3) cells would uncouple left and right sides but not grossly affect the frequency or internal dynamics of either oscillator. When this link was broken by hyperpolarizing the HN(3) cell on one side (Figs. 5, 7), the burst period of the cells on the other side increased 40–200% and their burst duration increased. Hence, reciprocal inhibition between the HN(3) cells must be important in timing oscillation in G3.

What stops burst of an HN(3) cell?

The termination of an HN(3) burst could normally result from 1) input from the contralateral HN(3) cell, 2) input from the ip-
FIG. 6. Hemilateral coordination in G3. A: chain including G1–G3 in which the HN(L, 3) cell had been photoinactivated. The experiment was analogous to those of Fig. 5 but here the untreated side of circuit was monitored. B: HN(2) and HN(3) cells contralateral to the inactivated cell remained coordinated in antiphase. Bursts of extra IPSPs, apparently caused by impulses in the ipsilateral HN(1) cell, occurred in phase with IPSPs induced by the HN(2) cell.

Thus the burst of the HN(1) and HN(2) cells appears to result from rather than cause the termination of an HN(3) burst.

What restarts burst of an HN(3) cell?

The previous section showed that the initiation of bursts in HN(3) cells appears to be the principal rate-determining step in the oscillation in G3. The recovery of an HN(3) cell could reflect 1) weakening input from the contralateral HN(3) cell, 2) weakening input from the ipsilateral HN(1) and HN(2) cells, or 3) an endogenous property of the HN(3) cell.

It is unlikely that the HN(3) cell normally recovers because of weakening input from the contralateral HN(3) cell. First, the impulse frequency of the contralateral HN(3) cell remains essentially fixed during most of the recovery phase. (In some preparations (e.g., Fig. 3) there was a decline in impulse frequency during the burst of an HN(3) cell, a feature that when present might contribute to oscillation (11, 19).) Second, the base-line potential (as defined in the legend of Fig. 8) of the contralateral HN(3) cell is constant during recovery of an HN(3) cell. Since the HN(3)-to-HN(3) synapse the base-line potential of the presynaptic cell determines the amplitude of the IPSP (18, 32), both the frequency and amplitude of IPSPs induced by the contralateral HN(3) cell should be
constant during recovery. Due to summation and the complication of other synaptic input it is impossible to measure precisely the amplitude of individual IPSPs during recovery but if anything, the IPSPs appear to increase in amplitude, as the potential of the recovering cell moves further from the reversal potential of the IPSP (Fig. 8). Thus there is no evidence that recovery results from a decline in either the frequency or the amplitude of IPSPs induced by the contralateral HN(3) cell.

The impulse frequency of the HN(1) and HN(2) cells does decline during the recovery phase of the ipsilateral HN(3) cell (Fig. 8A), suggesting that recovery might result from a decrease in synaptic input from the HN(1) and HN(2) cells. But recall that input from the contralateral HN(3) cell silences an HN(3) cell prior to the onset of HN(1) and HN(2)
activity (Fig. 8). If, as assumed, the input from the contralateral HN(3) cell remains constant, then that input should hold an HN(3) cell inactive irrespective of the activity of the HN(1) and HN(2) cells. (As the following paper (21) will discuss, input from the HN(1) and HN(2) cells can slow the recovery of an HN(3) cell and thereby affect the frequency of oscillation of the HN(3) pair.)

If input from the contralateral HN(3) cell does remain constant, then the only possible explanation for recovery can be that an endogenous process in an HN(3) cell makes it progressively more refractory to inhibition, thereby producing the phase transitions that constitute oscillation.

When one HN(3) cell is inactivated (Fig. 6), oscillation persists on the opposite side of the network but, predictably, the relationship among the remaining HN cells changes. Without contralateral input the burst of the remaining HN(3) cell apparently ends due to inhibition from the ipsilateral HN(1) and HN(2) cells rather than an endogenous process in the HN(3) cell, since initiation of the HN(2) burst precedes any slowing of the HN(3) burst (Fig. 6). In turn, the termination of the HN(2)’s burst follows the recovery of the HN(3) cell (Fig. 5). Thus the HN(1) and HN(2) cells cycle in alternation with the HN(3) cell, and again the driving force in the oscillation appears to be burst initiation rather than burst termination.

**HN cell processes in G4 form a local oscillator**

An isolated G4 includes the somata of the HN(4) cells plus segments of the HN(1), HN(2), and HN(3) pairs. Like the HN(3) cells, the HN(4) cells are linked by inhibitory synapses (18, 32) and they produce bursts in alternation (Fig. 9). An HN(4) cell normally receives inhibitory input from the ipsilateral HN(1) and HN(2) cells (3, 32). In an HN(4) cell in an isolated G4, there are two classes of IPSPs that are not matched by impulses in the contralateral HN(4) cell (Figs. 9, 10). These extra IPSPs, presumably caused by impulses initiated in the HN(1) and HN(2) axons (32), are sharply inhibited by an HN(4)
FIG. 9. Reciprocal inhibition in isolated G4. A: pairwise recording of the HN(4) cells. B: high-speed detail of A. Small unmatched IPSPs were caused by impulses arising in the severed axons of the HN(1) and HN(2) cells.

burst (Fig. 10). Hence there appears to be reciprocal inhibition between the HN(4) cell and the axons of the HN(1) and HN(2) cells in G4.

**Reciprocal inhibition between HN axons**

In an isolated G4 an impulse arising in the severed axon of an HN(3) cell can be detected by the IPSP it produces in the ipsilateral HE(4) cell (31; Fig. 2) or by the excitatory postsynaptic potential (EPSP) it produces in the ipsilateral HN(4) cell (32; Fig. 10). Under such conditions the axon of an HN(3) cell is active in phase with the ipsilateral HN(4) cell as it is when the G3 initiation site of the HN(3) cell is inactivated by current injection.

FIG. 10. An HN(4) cell inhibits its own inhibitory input. A: current was passed into both HN(4) cells, thereby blocking impulse activity. (The record of the HN(R, 4) cell was corrupted by noise and is not shown.) B: with the HN(R, 4) cell silent, synaptic input to the HN(L, 4) cell comprised IPSPs assumed on the basis of results obtained in full nerve cord preparations (3, 32) to be from HN(1) and HN(2) (marked by horizontal bars), alternating with EPSPs assumed to be from HN(3) and HN(X). At the indicated time a pulse of current induced a barrage of impulses, which inhibited and reset the burst of IPSPs. (The induced impulses are clipped.)
FIG. 11. HN(2) inhibits HN(3) in G4. A: intraganglionic processes of the HN(L, 3) and HN(L, 4) cells were photoinactivated in a chain of G1–G4. HN(L, 3) impulses were detected as the larger of the two classes of IPSP in the HN(L, 4) cell (Fig. 2). B: HN(2) impulses induced by a current pulse inhibited HN(3) activity. (The bridge was out of balance during the current pulse.)

FIG. 12. HN(3) inhibits HN(2) in G4. The preparation included G1–G4. Hyperpolarizing current (approximately 0.2 nA) was applied continuously to cell HN(L, 4). In most cases the attenuated spikes in the soma of the HN(L, 2) cell could be matched with IPSPs in the other two cells (solid lines). During an HN(L, 2) burst the HN(L, 3) cell was depolarized, as indicated in the current monitor trace (CM). The resulting HN(3) burst reset the HN(2) burst.
(6). HN(3) bursts remained coordinated with those of the ipsilateral HN(1) and HN(2) cells when the HN(4) cells were inactivated (Fig. 10), suggesting that there are synaptic connections within G4 among the HN(1), HN(2), and HN(3) axons. To test for inhibition from the HN(2) cell to the HN(3) axon in G4, the HN(L, 3) and HN(L, 4) processes were photoinactivated in G3 and G4, respectively (Fig. 11), leaving the interganglionic process of the HN(L, 3) cell intact, as monitored by the IPSP bursts in the HE(L, 4) cell. Under these conditions a volley of HN(L, 2) impulses silenced an HN(L, 3) burst (Fig. 11B).

A similar result was obtained for the HN(1) cell.

In order to test for inhibition of the HN(2) cell by the HN(3) axon, I hyperpolarized an HN(4) cell while recording from the ipsilateral IHN(2) and IHN(3) cells (Fig. 12). Such hyperpolarization causes the ipsilateral HN(2) cell to initiate impulses G4 (22), as shown in the present case in which an HN(2) impulse matched an IPSP the HN(4) cell and another about 20 ms later in the HN(3) cell. An induced HN(3) burst immediately silenced an HN(2) burst, and therefore the HN(2) cell (and presumably the HN(1) cell) is linked to

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**FIG. 13.** Reciprocal inhibition between the HN(4) cells produces side-to-side coordination in G4. **A:** in an isolated G4 the processes of the HN(3) cells normally produce bursts in alternation. **B:** the IIN(L, 4) cell in the preparation shown in **C** was photoinactivated. **C:** coordination between left and right sides of the network in G4 was destroyed by the inactivation of an HN(4) cell. IPSPs caused by HN(3) and HN(X) activity were present in both HE cells.
the ipsilateral HN(3) cell by reciprocal inhibition in both G3 and G4.

Reciprocal inhibition between HN(4) cells is necessary for side-to-side coordination in G4

If the minimal circuit for G4 (Fig. 13B) is complete then the interaction between the HN(4) cells should be necessary for side-to-side coordination, since it is the only link across the midline. With one HN(4) cell photoinactivated, the two sides of the circuit did in fact cycle independently (Fig. 13C). (In some preparations the photoinactivation of an HN(4) cell also silenced the G4 initiation site of the ipsilateral HN(3) cell, an effect possibly mediated by the electrical junction between the two cells although no dye coupling (29) was seen.)

Timing of oscillation in G4

G4 is more difficult to study than G3 because of the complicating residual activity of the HN(3) axons and because one cannot directly monitor HN(1), HN(2), and HN(3) activity. Apparently, the burst of one HN(4) cell normally terminates when it does because the burst of the other HN(4) cell begins, since when one cell's burst was blocked by hyperpolarization, the burst of the contralateral homologue extended slightly beyond its normal termination time (Fig. 14). The residual oscillation in G4 on inactivation of an HN(4) cell is more robust than that seen in G3 on inactivation of an HN(3) cell, and typically within a few cycles following hyperpolarization of an HN(4) cell a normal rhythm is restored. Two factors that might help account for this result are 1) the G4 initiation sites of cells HN(1) and HN(2) are more vigorous than their G3 counterparts (21), and this may lead to more balanced reciprocal inhibition between the remaining elements in G4 than in G3; and 2) reciprocal inhibition between the HN(1) or HN(2) axons and the HN(3) axons may strengthen the strong residual oscillation in G4.

Fig. 14. Inhibition helps terminate the burst of an HN(4) cell. A: in a chain including G4 and G5 HN(R, 4), activity was monitored by its IPSPs in the HE(R, 5) and HN(L, 4) cells. The HN(4) cells have secondary impulse-initiation sites in G5 (small squares), which become active when the primary site is inhibited (6, 31). (The IIE(5) cell also receives inhibition from the ipsilateral HN(3) and HN(X) (see Fig. 2). In the reduced preparation used in this experiment the three classes of IPSPs are in phase.) B: the HN(L, 4) cell was inactivated by current injection. (Impulse initiation continued from cell HN(L, 4)'s distal site in G5, but hyperpolarization of the HN(4) soma blocked the effect of that cell's synapse with the contralateral HN(4) cell (18.) On hyperpolarization of the HN(L, 4) cell, the HN(R, 4) burst was extended slightly (but consistently in numerous trials) in the cycles immediately following.
DISCUSSION

G3 and G4 contain matching oscillators

The heartbeat timer is an extended network with essential components distributed between G3 and G4. G3 and G4 behave as separate local control centers in the sense that each ganglion produces a reasonably normal rhythm when it is separated from the rest of the nervous system. In each ganglion the focus of oscillation is the reciprocal inhibition between the pair of HN cells originating in that ganglion and, in each, this interaction is the only link between left and right sides of the network. These main pairs of HN cells make identical connections with the axons of the HN(1) and HN(2) cells. The slight break in the symmetry of the system—the secondary initiation sites of the HN(3) cells in G4—is unimportant, since these sites are inactive when G3 is attached (6, 22, 31).

Other distributed neural networks, each ganglion of which can oscillate when phasic input from the rest of the nervous system is blocked, are the networks controlling leech swimming (27, 33) and crayfish swimmeret movements (12, 26).

Oscillation within subunits of timer

Reciprocal inhibition is the dominant theme in the G3 and G4 networks, and in every case cells linked in that fashion produce bursts in alternation. Yet it is clear that reciprocal inhibition does not by itself generate oscillation (8, 19). That is, if L and R are inherently active neurons that inhibit each other and if all properties of the neurons are time invariant, then the system will get stuck at one of two stable end points: L on and R off, or R on and L off. For the network to become an oscillator there must be a time-dependent process that undermines the stability of the two end points. The process could take one of two forms: 1) the inhibitory input to the inactive neuron could weaken with time (due to synaptic fatigue, for example) or 2) the inactive neuron could become increasingly refractory to inhibition due to a changing endogenous property. As shown in this paper, in the heartbeat oscillator, evidence supports the latter mechanism.

An endogenous process that would overcome inhibition has not been identified in HN cells, but it is possible that recovery is driven by a slow conductance change similar to those present in rhythmically active neurons in other systems. Yet, unlike the oscillatory neurons of mollusks (10), for example, which hyperpolarize spontaneously to complete the burst cycle, an HN(3) or HN(4) cell normally hyperpolarizes due to inhibition from the contralateral homologue. It is not known if the HN cell would eventually hyperpolarize in the absence of synaptic inhibition, since it is impossible to block all input without changing the cellular environment markedly (see below). However, whether the capacity to hyperpolarize spontaneously is present in the HN cells or not, that property is not strongly expressed in the cycle and it is, therefore, of limited dynamic importance.

The intrinsic properties of rhythmically active neurons often depend critically on exogenous factors (2). For example, an increase in the level of proctolin causes the normally stable membrane potential of the cardiac motor neurons of the lobster to oscillate spontaneously (15). Similarly, the cellular properties of neurons in the stomatogastric ganglion of the lobster change when inputs from other ganglia are stimulated (1, 23, 24). It is possible, therefore, that with the appropriate input the tendency for an HN cell to hyperpolarize spontaneously may increase to the point that it contributes to the oscillation of the network. An indication that such a property is latent in the HN cells is their behavior when C1 is removed from the bathing medium (4). The removal of C1 blocks inhibition within the leech CNS, thereby greatly increasing spontaneous activity, including that of the HN cells. All HN cells (except the HN(5)s), including those that show little or no spontaneous rhythm in normal saline (the HN(1) and HN(2) cells (Fig. 5) and the HN(6) and HN(7) cells (unpublished results)), oscillate at high frequency in low-C1 saline. The oscillation of the HN cells may be stimulated directly by the removal of C1 or indirectly by the disinhibition of neurons releasing modulators of the HN cells.

Thus the oscillation of the HN network apparently stems from a balance between a relatively fixed component, the synaptic connectivity, and a potentially variable compo-
neural circuit timing heartbeat in leech 625

teent, the membrane properties of the HN(3) and HN(4) cells.

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REFERENCES


