

Rate modification in the heartbeat central pattern generator of the medicinal leech

Edmund A. Arbas and Ronald L. Calabrese

Biological Laboratories Harvard University 16 Divinity Ave. Cambridge, Massachusetts 02138, USA

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Summary. 1. Intracellular recordings from neurons in partially dissected leeches and isolated nerve cords were used to study the effects of temperature, sensory stimulation and locomotory activity on the output of the central pattern generator that drives heartbeat.

2. The rate of motor burst production by the heartbeat oscillator changes on gradual heating or cooling with a Q_{10} averaging around 2.4. Abrupt cooling of isolated nerve cords can induce additional changes in heart rate that are associated with 'paradoxical' firing of motor neurons.

3. Heart rate was accelerated when mechanical stimuli were applied to the body wall of partially dissected preparations or when restrained preparations spontaneously initiated movements. For instance, marked acceleration of heart rate occurred when preparations exhibited body movements and motor neuron activity characteristic of swimming in intact animals. Activation of the motor program for swimming in isolated nerve cords led to acceleration of heartbeat oscillator cycling. Accelerations of heart rate associated with swimming are therefore mediated at least partially through central interactions.

4. Individual identified neurons were tested for their influence on the cycle rate of the heartbeat oscillator in isolated nerve cords. Activity of individual mechanosensory neurons was found to accelerate heart rate. Touch mechanosensory neurons were also found to influence the heartbeat oscillator through a rapidly adapting inhibitory pathway. Activity of individual motoneurons generally did not affect heart rate. Some interneurons and neuroeffector cells known to cause motor activation (e.g. swim initiating interneurons, serotonergic Retzius cells) can also produce acceleration of heart rate.

Introduction

Central networks of neurons program most rhythmic movements (see Delcomyn 1980; Roberts and Roberts 1983 for reviews). The rhythmicity of such networks derives partly from oscillatory properties of individual neurons within them and partly from the pattern of their synaptic connections (see Roberts and Roberts 1983). Certain properties of these networks can be modified by extrinsic inputs such as sensory feedback or inputs from other neural centers so that the motor acts produced are altered to match the changing needs of the animal (Grillner 1975; Selverston and Miller 1980; Miller and Selverston 1982a, b; Sigvardt and Mulloney 1982; Nagy and Dickinson 1983; Dickinson and Nagy 1983). The identification and characterization of modifiable properties of pattern generators and the pathways that control them are necessary for a complete understanding of the neural basis of rhythmic behavior.

Our studies have focussed on the heartbeat of the medicinal leech, *Hirudo medicinalis*, a simple motor act whose neural substrates have been characterized in cellular detail (Stent et al. 1978; Calabrese and Peterson 1983). We have addressed the question: "How does the leech heartbeat vary in the course of the animal's normal behavior, and what pathways control it?". We report here the effects on heart rate of changing temperature, of sensory stimulation, and of neural activity associated with locomotion.

Heartbeat of the leech

Rhythmic constrictions of muscular vessels, the 'heart tubes' or 'hearts', move blood in the closed circulatory system of the leech. Heart muscle cells

exhibit oscillatory properties that contribute to heart beating (Maranto and Calabrese 1984b), but the rhythm and segmental coordination of heart tube constrictions is under the control of a neural network within the central nervous system (CNS) (Stent et al. 1978; Calabrese and Peterson 1983). This network continues to produce the motor pattern for heart tube constriction in isolated nerve cords, and hence, constitutes a central pattern generator.

Elements of the heartbeat pattern generator

Motor neurons. A bilateral pair of heart motor neurons, the HE cells, are located in the 3rd through the 18th ganglia and are indexed according to body side and ganglion number from HE(L,3) to HE(R,18) (Thompson and Stent 1976b; Shafer and Calabrese 1981) (Fig. 1). Each HE cell directly innervates the ipsilateral heart tube in its own segment (Maranto and Calabrese 1984a). HE cell activity is organized into rhythmic bursts of action potentials interrupted by barrages of inhibitory postsynaptic potentials (ipsp's). Action potentials in the HE cells produce unitary excitatory junctional potentials in heart muscle (Thompson and Stent 1976a), cause constriction of the heart tubes in their own segments and entrain the heart tube's inherent constriction rhythm to match the period of HE cell cycling (Maranto and Calabrese 1984b). Both the pattern of bursting of individual HE cells, and their coordination along the body are maintained in isolated nerve cords and these match the constriction patterns observed in innervated heart tubes (Calabrese and Peterson 1983).

HE cell firing is structured into bursts by rhythmic inhibition from a network of interneurons, the HN cells (Thompson and Stent 1976b; Calabrese 1979; Calabrese and Peterson 1983).

Heart interneurons. A bilateral pair of HN cells is located in each of the first seven ganglia of the nerve cord (Thompson and Stent 1976b; Shafer and Calabrese 1981) (Fig. 1). The rhythm of the network is determined by the subset of HN cells in the first 4 ganglia through a blend of their inherent membrane properties and their reciprocal inhibitory connections. They have been termed the 'timing oscillator' of the network. (For a detailed description of timing oscillator operation see Peterson and Calabrese, 1982 and Peterson 1983a, b.) All stable changes in the timing of network cycling must be mediated through the agency of these HN cells.

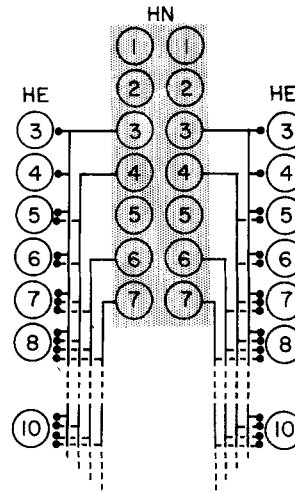


Fig. 1. Circuit diagram showing inhibitory synaptic connections from identified HN interneurons to HE motor neurons. Open circles represent cells (each identified by the number of its ganglion), lines represent their processes, while filled circles denote inhibitory chemical synaptic connections. Note that recording from posterior HE cells permits the monitoring of the activity of the entire ensemble of HN cells

Materials and methods

Animals, apparatus and recording techniques. Leeches, *Hirudo medicinalis*, were obtained from commercial suppliers and maintained at 15 °C in aquaria for up to 6 months prior to use. Intracellular recording and stimulation techniques have been described previously (Thompson and Stent 1976a), and these were supplemented with extracellular recordings from nerve roots using glass-tipped suction electrodes. HE and HN cell somata could be identified unambiguously by their positions in ganglia and by their characteristic impulse burst pattern. All preparations were bathed in physiological saline (Nicholls and Baylor 1968) which was changed frequently.

Since HE cell motor bursts from the CNS entrain any rhythmicity inherent in heart muscle cells, their timing provides a faithful measure of the timing of heart tube constriction. Therefore, throughout this report, phrases such as 'heart rate' or 'rate of heartbeating' will be used interchangeably with 'rate of heartbeat pattern generator cycling'. Other nomenclature is standard (Payton 1981), and labelling of nerve roots follows the system described by Ort et al. (1974).

Preparations and procedures. Recordings were made from isolated nerve cords as well as partially dissected preparations. In all cases, activity of the heartbeat oscillator was monitored by intracellular recording from HE or HN cells. Many movements such as shortening and bending are produced through activity of longitudinal muscle motorneurons, the L cell, and dorsal excitor cell 3 (Nicholls and Purves 1970; Kristan 1982). Both of these neurons have axons in the dorsal posterior (DP) nerve and extracellular recordings of the activity in their axons were used in all preparations as an indicator of motor activity. In partially dissected preparations, ganglia 9 through 15 were dissected and pinned out for intracellular recording from HE cells, while segments anterior to the 8th were left intact. The movements of the only partially restrained anterior segments were observed through a dissecting microscope and recorded on the voice channel of a tape recorder while monitoring output of the heartbeat system. In some cases, the connectives between

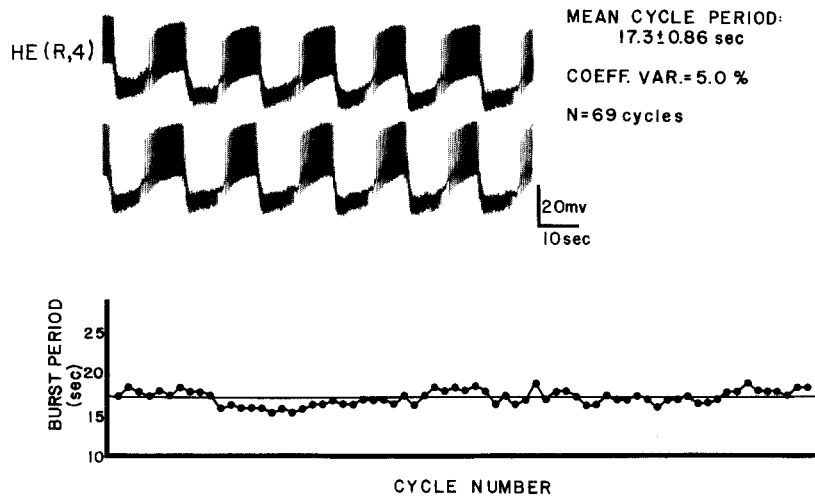


Fig. 2. Cycling of the heartbeat CPG can be very stable in isolated nerve cords. Upper panel shows two segments of a continuous intracellular recording of an HE (R,4) cell. Burst period is plotted against cycle number below for many more cycles of the same recording. Solid line represents the mean period of 17.3 s

the 1st and 2nd segmental ganglia were also cut to remove the influence of the 'head-brain'. The effects of changing temperature on heart rate were studied in nerve cords isolated from leeches acclimated to 15 °C. Lengths of nerve cord of up to 8 ganglia (G2–G10) were pinned in a dish of 80 ml volume with a thermometer immersed in the bathing solution alongside the ganglia. Temperature was read from the thermometer at frequent intervals. Temperature was changed 'gradually' or 'abruptly' while recording from an HE cell by substituting Ringer solution in the preparation chamber with Ringer solution heated or cooled to the desired temperature in a water bath. To change temperature gradually, small aliquots of bath saline were exchanged for heated or cooled saline every 30 s. When cooling, a peltier cooling device was activated; when heating, it was deactivated. By these means, temperature changes of several degrees per minute were achieved. Abrupt temperature changes were made by removing about 50 ml of bath saline and replacing it with a single aliquot of heated or cooled saline that was added to the recording chamber within 15 s (see Fig. 4). Exchanging bath saline by either method with saline of identical temperature produced no changes in period of the heartbeat oscillator. Temperature coefficients were calculated as $Q_{10} = (K_2/K_1)^{10/(T_1 - T_2)}$, where K_1 and K_2 are burst periods corresponding to the higher temperature T_1 , and the lower temperature, T_2 , respectively (Prosser 1973, p. 363).

The influence of individual neurons on heartbeating was assessed in the following way. Lengths of nerve cord of two to thirteen ganglia (G2 through G15) were pinned in a chamber with a volume of 2.5 ml. Output of the heartbeat oscillator and nerve cord activity were monitored as described above, and an intracellular electrode was also placed in the soma of a test neuron. Five to ten cycles of stable activity were recorded from the HE or HN cell, then current pulses of different amplitudes were injected into the test neuron and changes in heart rate and DP nerve activity noted. The influence of a given test cell was ranked negative when repeated changes in polarization and firing due to injected current consistently failed to affect the heartbeat oscillator and positive when heart rate was influenced repeatedly over several trials in several preparations. The influence of test cells was ranked questionable when changes in HE or HN cell cycling that appeared to be related to electrical manipulation of a test cell were not repeatable over several trials or in separate preparations.

Injury discharge in test neurons caused by electrode penetration sometimes led to prolonged acceleration of heart rate and increases in DP nerve activity in previously quiescent nerve

cords. In these cases, and in certain 'active' preparations where the heart rate was high, manipulation of test cell activity failed to show further influence on the rate. Furthermore, this saturation effect was observed in some preparations where numerous test cells were sampled, and even neurons known to affect heart rate from other experiments often failed to affect rate.

Results

Stability of the heartbeat rhythm and the effects of temperature

Rate of heartbeating can be very stable in unstimulated isolated nerve cords maintained at constant temperature, with HE cell burst periods exhibiting a coefficient of variation as low as 5% over long recordings (Fig. 2). Similar results have been previously obtained with recording made directly from timing oscillator interneurons (Calabrese 1980).

Manipulation of bath temperature altered the heart rate; heating induced acceleration, cooling led to deceleration. HE cell burst periods varied over a range from 4.5 s at 26 °C to almost 60 s at 4 °C in different preparations. The response of a single preparation to gradual cooling from 25 °C to 4 °C is plotted in Fig. 3 where each point represents the mean HE cell burst period over 10 cycles at each temperature. The response to gradual temperature change exhibited an average Q_{10} of about 2.2 over a range from 7 °C to 25 °C in this preparation.

All preparations were made from leeches acclimated to 15 °C, but not all preparations exhibited the same rates at similar temperatures (Table 1). Additionally, changes in the rate-temperature relationship of heartbeating were observed when ongoing activity recorded from DP nerves also changed. In some instances DP activity increased spontane-

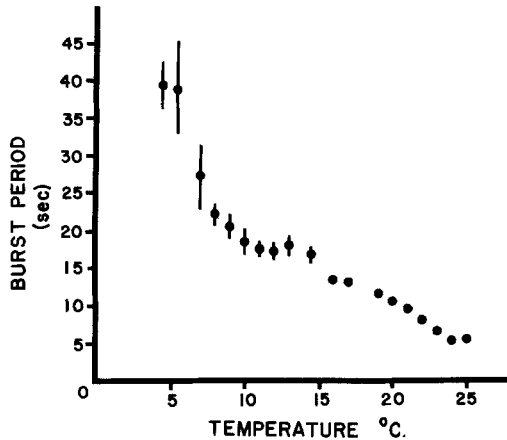


Fig. 3. Rate-temperature relationship of the heartbeat oscillator during gradual cooling from 25° C to 4° C. The preparation consisted of isolated ganglia 3 and 4. Each point represents the mean period of 10 bursts recorded intracellularly from an HE (R,4) cell at each temperature. Bars, standard deviations. At temperatures above 15° C these were smaller than the diameter of the plotted points

ously; in others it appeared to be altered by abrupt temperature changes. Figure 4 shows examples of these types of changes observed in a preparation consisting of isolated ganglia 2 through 8. Initially the heartbeat oscillator was cycling with a mean burst period of 13.3 s at 16° C, as indicated by an intracellular recording from HE(R,5) (Fig. 4A). A large influx of cold Ringer solution into the preparation chamber (arrow) brought the preparation to 12° C abruptly and the burst period lengthened to a mean of 18.8 s giving a Q_{10} of 2.4. There was no appreciable change in DP activity over this temperature transition. The same preparation continued cycling with an 18.8 s average period at 12° C (Fig. 4B). The temperature was brought abruptly to 10° C (arrow). At this temperature transition, there was a sudden increase in DP nerve activity. The mean HE cell burst period at 10° C became 18.3 s, indicating a slight acceleration of oscillator cycling on cooling. Ignoring the first two HE cell cycles after the transition (11.5 s and 15.5 s periods respectively) as representing transient instability, the mean HE cell burst period over the following 10 cycles was 19.3 s. This second calculation indicates an overall deceleration on cooling, yet the Q_{10} of 1.1 for the transition between 12° C and 10° C is still low. This low value is likely due to the superposition on the heartbeat oscillator of an acceleratory influence that is related to the onset of activity in motoneurons as recorded in the DP nerve (see later sections). As shown in Fig. 4C, the same preparation was cycling at a mean period of 18.6 s (note the change

Table 1. Rate-temperature relationship of the heartbeat oscillator subjected to gradual temperature changes

Prep.	K_1	K_2	T_1	T_2	Inc./ Dec.	Q_{10}
1 a.	9.7 + or - 0.7	21.7 + or - 2.4	20	12	▼	2.7
b.	9.4 + or - 0.5	21.7 + or - 2.4	20	12	▲	2.9
2 a.	15.6 + or - 1.7	26.1 + or - 0.8	20	15	▼	2.8
b.	26.1 + or - 0.8	35.4 + or - 2.5	15	10	▼	1.8
c.	16.1 + or - 0.9	35.4 + or - 2.5	20	10	▲	2.2
d.	11.5 + or - 0.7	16.0 + or - 0.9	25	20	▲	1.9
e.	11.5 + or - 0.7	19.0 + or - 0.8	25	20	▼	2.8
f.	19.0 + or - 0.8	41.6 + or - 3.3	20	10	▼	2.2
g.	11.1 + or - 1.1	41.6 + or - 3.3	25	10	▲	2.4
h.	11.1 + or - 1.1	25.9 + or - 2.9	25	15	▼	2.3
3 a.	6.5 + or - 0.4	10.7 + or - 0.8	20	15	▼	2.7
b.	10.6 + or - 0.7	28.5 + or - 3.6	20	10	▼	2.7
4 a.	22.6 + or - 0.8	26.8 + or - 1.3	10	8	▼	2.4
b.	17.6 + or - 0.8	26.8 + or - 1.3	14	8	▲	2.0
c.	13.1 + or - 0.8	17.6 + or - 0.8	17	14	▲	2.7
5 a.	13.3 + or - 1.3	19.3 + or - 1.4	16	12	▼	2.5
Mean						
$Q_{10} = 2.4 \pm 0.3$						

▲ indicates that temp. was increased from T_2 to T_1
▼ indicates that temp. was decreased from T_1 to T_2

$Q_{10} = (K_2/K_1)^{10/(T_1-T_2)}$, where K_1 and K_2 represent the period at the higher temperature, T_1 , and the lower temperature, T_2 , respectively. Results are shown for different temperature transitions (lettered) in five preparations (numbered)

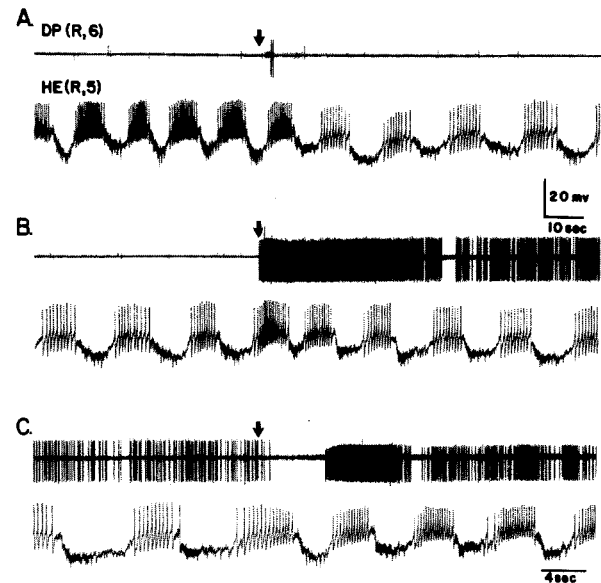


Fig. 4A-C. Changes in heartbeat caused by abrupt cooling or heating. All recordings are from a preparation consisting of a chain of six isolated ganglia (G2-G8). Temperature was changed by pouring cooled (A, B) or heated (C) Ringer solution into the preparation chamber. Arrows, time at which addition of Ringer began. See text for further explanation. A and B are at the same scale. Note change in time scale in C

in time scale) at 11 °C when abrupt warming (arrow) brought the preparation to 18 °C and a mean burst period of 10.5 s, yielding a Q_{10} of 2.3. DP nerve activity showed a transient pause on heating but remained high after the temperature change. Similar pauses in DP activity were observed on other occasions when the preparation was warmed. As shown in Fig. 4B and C, the DP activity brought on with cooling was usually long lasting and was accompanied by pronounced lowering of the Q_{10} for heartbeating over the temperature transition where DP activity was initiated. The abrupt cessation of DP firing on warming of the preparation was usually of short duration and accompanied by little or no change in the Q_{10} for heartbeating.

Rate of heartbeating changes with behavioral state

The stable cycling of the pattern generator usually observed in unstimulated nerve cords at constant temperature represents its output in isolation from the sensory feedback and behavior-associated neural activity normally present in the intact animal. To examine the output of the pattern generator under conditions that permit this type of neural activity, recordings from isolated nerve cords were compared to those made from partially dissected preparations.

Three parameters were monitored in partially dissected preparations: (1) heart rate was monitored with intracellular recordings from HE cells of posterior ganglia; (2) motor activity other than that related to heart tube constrictions was monitored extracellularly from DP nerves; and (3) movements of the anterior segments were observed visually through a dissecting microscope and recorded on the voice channel of a tape recorder.

Fluctuations in the rate of heartbeating were found to be associated with the movements of undissected anterior body segments and increases in discharge of the monitored DP nerve. Rate changes occurred when animals moved spontaneously, or when tactile stimuli were applied to the skin. These changes took the form of sudden increases in heart rate each followed by a gradual return to a basal level during times of inactivity.

Marked acceleration of heartbeating was associated with onset of swimming motor activity which was assayed by observing swim-like undulations of undissected anterior body segments and recording rhythmic discharges of motoneurons from DP nerves (Fig. 5). In one preparation all nerves to the head-brain were cut through a small incision in the head, but other anterior ganglia

were left intact. This preparation chronically swam for over an hour while HE cell recordings were made. The mean HE cell cycle period was maintained at about 9 s (8.98 ± 0.05 , $n = 169$ cycles) during swimming (Fig. 5). The motor program for swimming could be transiently interrupted by applying a sharp mechanical stimulus to the skin which elicited shortening (Fig. 5A). Shortening was followed by a period several tens of s long in which the body slowly relaxed and motor axons in DP nerves were quiet, after which the anterior body flattened and swimming resumed. The HE cell burst period lengthened gradually to different levels during such quiet times, and then shortened again as swimming resumed (Fig. 5A, B). Mean HE cell cycle period during the quiet times was 10.52 ± 0.13 s ($n = 79$ cycles), a period 17% longer than that maintained during swimming. The sample of HE cell burst periods measured during quiet periods was found to be significantly different at the 0.1% confidence level from those measured during swimming by applying the Wilcoxon two sample test (Sokal and Rohlf 1969 p. 393).

Are period changes centrally or peripherally mediated?

Swimming motor activity produced by isolated nerve cords can be unequivocally identified by the characteristic pattern of action potentials recorded from DP nerves, and can be easily activated in a number of ways (Kristan and Calabrese 1976; Weeks and Kristan 1978; Willard 1981). Thus, it was possible to determine the relationship between changes in rate of heartbeating and motor activity during swimming.

Heartbeat oscillator cycling was monitored by intracellular recordings from HE or HN cells in isolated nerve cords consisting of the anterior brain and first sixteen segmental ganglia. The motor program for swimming was activated by stimulating peripheral nerve roots (Kristan and Calabrese 1976), by stimulating serotonergic neurons (Willard 1981) or by intracellular stimulation of swim-initiating interneurons, cells 204 (Weeks and Kristan 1978). Activation of the motor program for swimming by any means was accompanied by accelerations of heartbeating indicated by shortening of HE or HN cell burst periods. Figure 6 shows recordings from a preparation in which swimming motor pattern was induced in an isolated nerve cord by depolarizing a swim-initiating interneuron, cell 204, in ganglion 10 while recordings were made from timing oscillator interneuron, HN (L,4). The

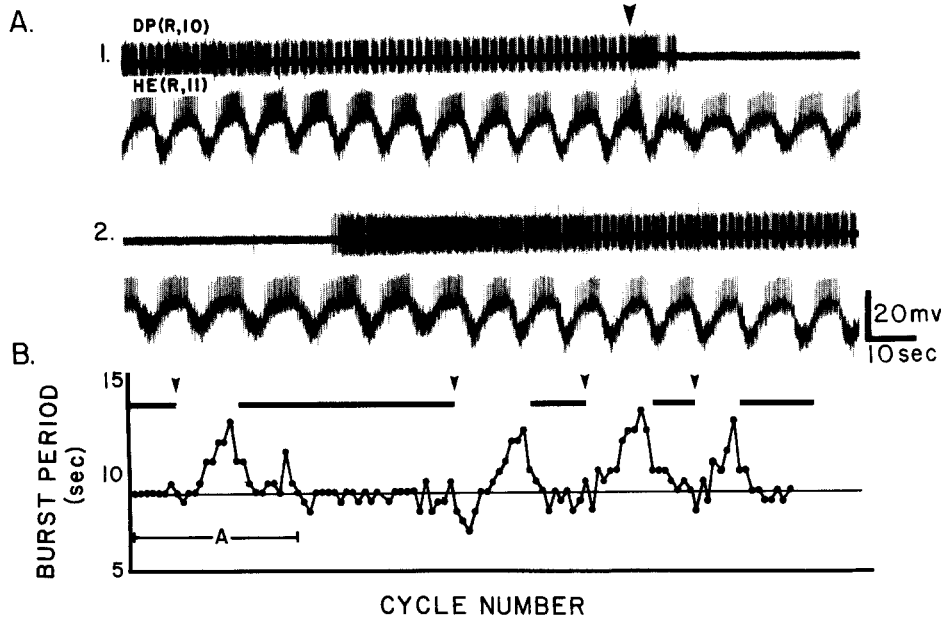


Fig. 5A, B. Heartbeat accelerates when the swimming motor program is expressed in a partially dissected preparation. It slows when swimming is interrupted. A1 and 2: Continuous recordings from DP nerve of segment 10 (upper traces) and an HE cell (R,11) (lower traces) in a chronically swimming preparation. The swimming motor program was transiently interrupted by applying a mechanical stimulus to the skin of the undissected anterior segments (A1, arrow) and eliciting shortening. After a quiet period, the anterior body relaxed, elongated, flattened and spontaneously began to produce swim-like undulations accompanied by rhythmic discharge in the DP nerves (A2). **B** Plot of burst period of the HE cell vs cycle number over about 35 min of continuous recording from the same preparation. The heavy line at the top marks times in which the motor program for swimming was active. This was transiently interrupted as described above four times (arrows). The solid line indicates the mean cycle period of the HE cell during swimming recorded over 167 cycles (= 8.98 s). The plot from recordings of A1 and A2 is underlined

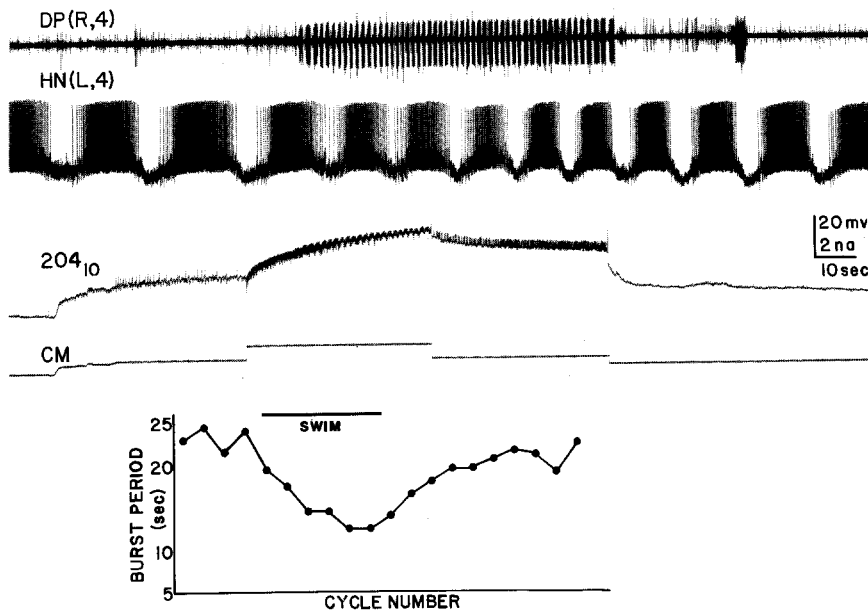


Fig. 6. Acceleration of heartbeat associated with swimming is largely centrally mediated. Heartbeat oscillator output was monitored by intracellular recording from cell HN (L,4) (2nd trace) in an isolated nerve cord. Swimming motor activity was induced by depolarizing swim-initiating interneuron 204 in ganglion 10 (3rd trace). As bursts appeared in the DP nerve (upper trace), cycling of cell HN (L,4) accelerated. The burst period of the cell is plotted against cycle number below, with the activity of the motor program for swimming indicated by a bar. CM, current monitor

HN cell burst period was reduced by about 48%, from 24 s to 12.5 s, during the bout of swim motor activity.

These data indicate that there are strong central interactions between motor networks for behavior such as swimming and for heartbeat. Changes in

rate of heartbeating associated with locomotor behavior can be mediated by central interactions between motor circuits and do not require sensory feedback that might be provided by movement or by buildup of an oxygen debt due to muscular activity.

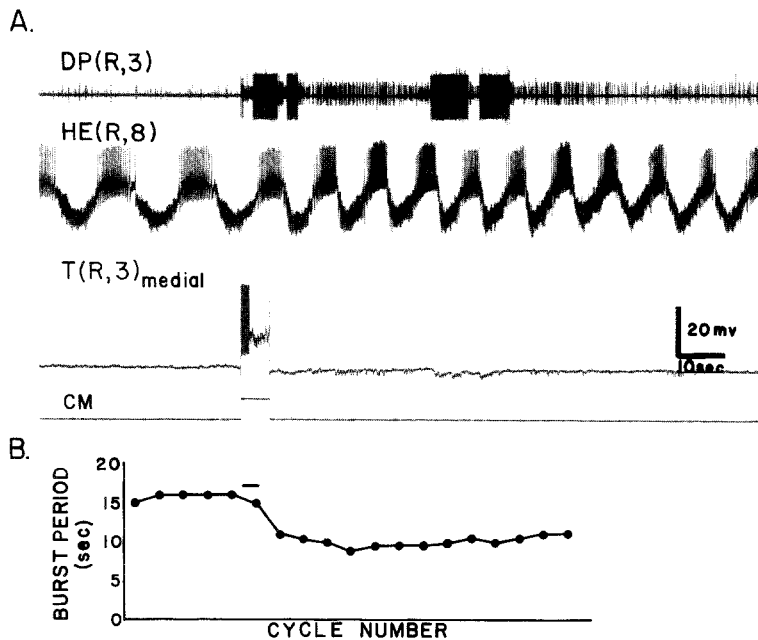


Fig. 7A, B. Activity of identified sensory cells can effect changes in heartbeat. **A** Stimulation of a medial 'touch sensory' cell in ganglion 3, T (R,3), (3rd trace) by current injection led to acceleration of cell HE (R,8) cycling and a concomitant increase in activity of motor units recorded in the DP nerve. CM, current monitor. **B** HE burst period plotted against cycle number with stimulus to the T cell marked by a bar

Influence of activity in identified neurons

It has been shown in previous sections that changes in heart rate can be effected through a variety of pathways with the common feature that activity of motor neurons to body wall muscles is also stimulated. For example, mechanical stimuli to the skin that produce shortening or other movements in previously quiescent preparations also affect heart rate. Such stimuli applied to intact leeches produce a variety of motor responses including shortening, local or whole-body bending and swimming (Kristan et al. 1982) depending on the location of the stimulus on the body. The input that triggers shortening or bending is mediated by the touch, pressure and nociceptive ('T', 'P', and 'N' respectively) mechanosensory neurons (Nicholls and Baylor 1968; Kristan 1982; Kristan et al. 1982).

Sensory neurons. Stimulation of individual T, P, or N mechanosensory neurons by injected current produced accelerations of heart rate that often outlasted the stimulus duration (Fig. 7, Table 2). This acceleration was accompanied by activation of motor axon firing in DP nerves similar to that observed by Kristan (1982). The ability of mechanosensory neurons to accelerate heart rate is less robust in single isolated ganglia or pairs of connected ganglia than in longer pieces of nerve cord containing many ganglia.

Paired intracellular recordings revealed an inhibitory pathway from T cells to HN cells. A burst of action potentials in any T cell in ganglion 3

or 4 leads to a short lasting inhibition of the ipsilateral HN cell in the same ganglion and a weaker inhibition of the HN cell one ganglion away (Fig. 8). This pathway appears to be polysynaptic since ipsp's recorded in HN cells do not match T cell spikes.

Inhibitory pathways from T cells to HN cells appear to be restricted to ganglia 3 and 4. Activity of T cells in ganglion 3 influences HN cells 3 and 4. T cells of ganglion 4 influence the same two HN cells. Activity of T cells in ganglia 1, 2, 5, 6 and 7 does not appear to influence firing of HN cells by similar inhibitory pathways. Similarly, T cells of ganglia 3 and 4 do not appear to influence HN cells 5, 6 or 7 by an inhibitory pathway of this type. Effects of T cells on HN cells of ganglia 1 and 2 have not been tested. Under appropriate conditions, properly timed bursts of T cell spikes might entrain the rhythm of the heartbeat system through their action on the timing oscillator of the heartbeat system. This pathway of influence does not, however, account for the acceleration in heart rate described above. Since behavioral conditions under which T cells might fire phasically are not known, the functional significance of this inhibitory pathway is not readily apparent.

Motor neurons. Intracellular stimulation of identified motoneurons to a number of body wall muscles and the hearts (the HE cells) failed to affect the rate of heartbeating. The exception among motor neurons was the excitor of dorsal and ventral longitudinal muscles, the L cell (Stuart 1970; Ort

Table 2. Identified neurons found to have influence on heart rate^a

Name	Function	Effect	Comments
<i>Sensory neurons</i>			
T1,T2,T3	Mechanosensory/ Touch	+/-	Heart rate changes accompany motor activation of DP. Inhibitory path to timing oscillator (see text)
P1,P2	Mechanosensory/ Pressure	+	Heart rate changes accompany motor activation of DP
N1,N2	Mechanosensory/ Nociceptive	+	Heart rate changes accompany motor activation of DP
<i>Motor neurons</i>			
L	Excitor of dorsal and ventral longitudinal muscles	?	Influence variable (see text)
<i>Identified interneurons</i>			
HN cell 1-4	Elements of heart beat timing oscillator	+	All can reset and entrain network rhythm (Peterson and Calabrese 1981)
204, 205	Swim-initiating interneurons	+	Activate swim bursts and/or increased root activity accompanied by acceleration of heartbeat oscillator
<i>Neuroeffector cells</i>			
Retzius	Serotonergic giant cell	+	Acceleration of heartbeat oscillator/increased root activity, swim bursts
Leydig	Function unknown	?	Activity leads to transient pauses in bursting

^a Names and functions after Muller et al. (1981) appendix D. + indicates acceleratory influence; - indicates deceleratory/inhibitory influence

et al. 1974) which weakly influenced heart rate in some but not all preparations.

Premotor interneurons. We tested premotor interneurons for their effects on heartbeat. Most of them including the S cell, had no influence on heart rate. As presented earlier, stimulation of swim-initiating neurons accelerates heartbeat rate, but these do not appear to make direct synaptic contacts with interneurons of the heartbeat timing oscillator.

Neurons that modulate motor rhythms. In addition to premotor interneurons that exert their influence on motor rhythms through conventional fast-acting synaptic pathways, certain neurons in leech CNS have been shown to exert their influence through the release of blood-borne neurohumors. Most notably, the large Retzius cells release 5-hydroxytryptamine (5-HT) and increase the probability of spontaneous episodes of swimming (Willard 1981). Figure 9 shows recordings from a preparation where Retzius cells in ganglia 3 and 4 were stimulated chronically for three min. This treatment led to acceleration of heartbeating and concomitant bursts of motor activity recorded in DP nerves, including bouts of swimming motor activity as described by Willard (1981).

Activity in the electrically coupled Leydig cell network (Keyser et al. 1982) produced by intracellular current injection was found to cause transient pauses in HE cell firing (Arbas and Calabrese 1983). These pauses last from tens of seconds to nearly a minute depending on the intensity of Leydig cell firing. Details of the action of Leydig cells on the heartbeat oscillator will be presented in a separate report.

Discussion

Heart rate of all animals varies greatly with exercise and rest, and is adjusted to meet circulatory

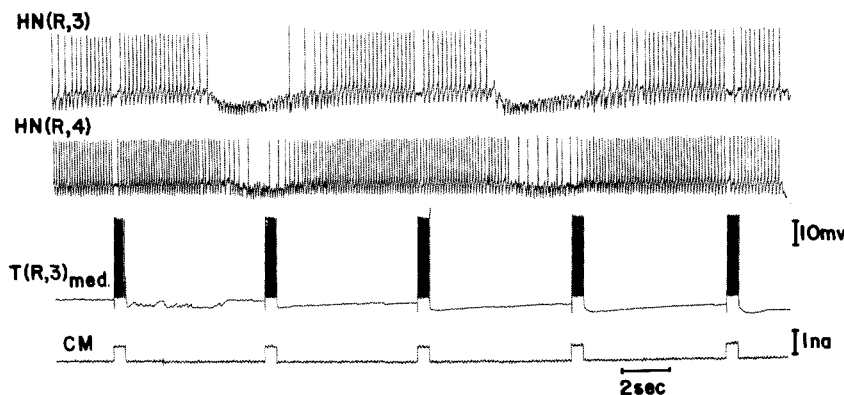


Fig. 8. Demonstration of inhibitory pathway from T cells to HN cells. When a single T cell of ganglion 3 was made to fire by intracellularly injected current (CM current monitor, 4th trace), ipsp's were generated in ipsilateral HN cells of both ganglia 3 and 4, producing a pause in the firing of action potentials during the normal HN cell bursts. The influence of this pathway seems to be stronger in the same ganglion as the stimulated cell than in adjacent ganglia (e.g. compare the effect in both HN cells in the middle burst)

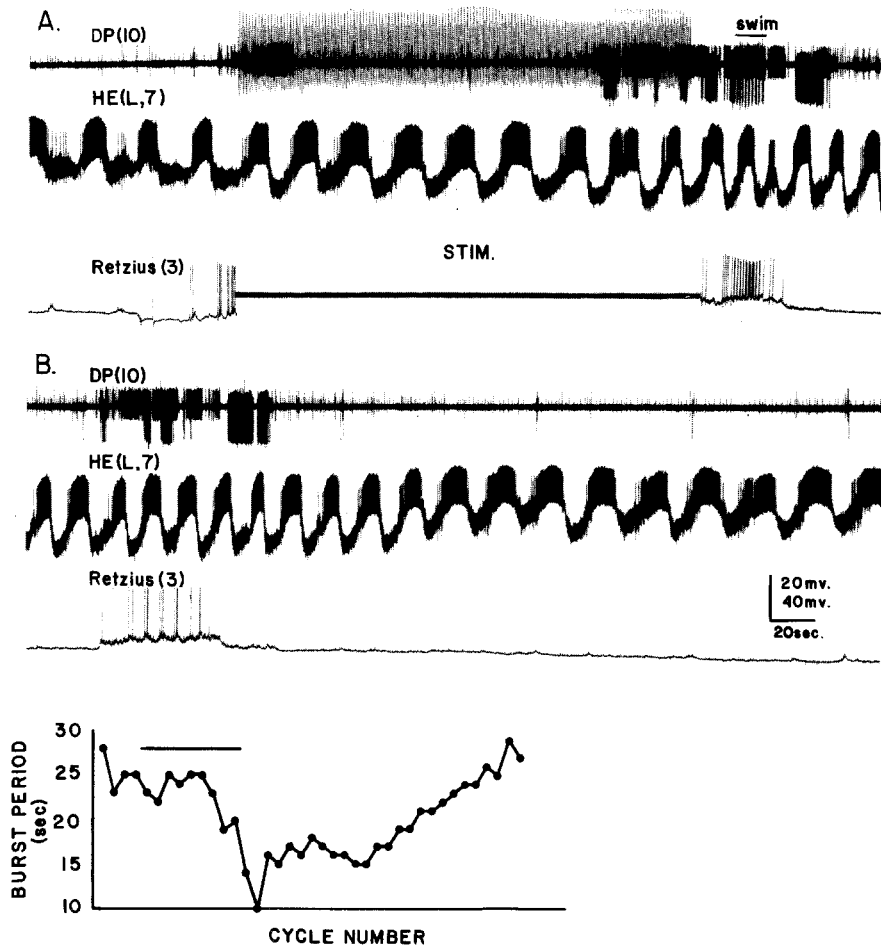


Fig. 9A, B. Changes in heartbeat are associated with stimulation of serotonergic neurons. A, B Continuous recordings from DP nerve 10 (upper traces), cell HE (L,7) (middle traces) and Retzius cell of ganglion 3 (Retzius cell of ganglion 4 also recorded but not shown). One Retzius cell in each of ganglia 3 and 4 was made to fire by current injection (700 ms duration depolarizing pulses at 1 Hz for 3 min, average spike frequency during pulses about 17 Hz) indicated by solid line in A (also note stimulus artifacts in DP nerve recording). This treatment led to acceleration of HE cell cycling and concomitant bursts of motor activity in DP nerve (including one bout of the swim motor pattern, A). HE cell burst period for this sequence is plotted against cycle number below, with the duration of stimulus to the Retzius cells shown by a bar

needs through a variety of complex reflexes (Prosser 1973). Neural regulation of heartbeating has been demonstrated directly in both neurogenic and myogenic hearts of invertebrates (Maynard 1953; Larimer and Tindell 1966; Watson and Wyse 1978; Hartline 1979; Dieringer et al. 1978; Koester et al. 1979; Koch and Koester 1982) as well as in vertebrates (Prosser 1973; Sagawa et al. 1974). In the present study, we have shown the influence of temperature and of behaviorally relevant neural activity on the rate of heartbeating in the leech.

Effects of temperature

Output of the the heartbeat oscillator of the leech follows gradual changes of temperature with a Q_{10}

that approaches or falls into the range typical for most metabolic processes, 2.0–2.5 (Prosser 1973) (Table 1). Abrupt cooling of the nerve cord that also triggers the onset of activity in nerve roots can lead to a sudden increase in the rate of heartbeating which is superimposed on the normal rate-temperature relationship.

The changes in DP nerve activity observed with abrupt cooling or heating of the nerve cord are qualitatively similar to the 'paradoxical' activity changes recorded from nerve cords of a number of invertebrates (slugs, roaches, crayfish, Kerkut and Taylor 1956; earthworms, Laverack 1961), as well as from particular sensory cells (crayfish stretch receptor, Van Gelder and Krnjevic 1961; Winter 1973; mammalian muscle spindle receptors, Lippold et al. 1960). In all of these systems,

cooling leads to transient increases in activity, heating to transient decreases. These activity changes have been termed paradoxical because they are opposite in sign to the usual effects on rate of cooling or heating. The functional significance of paradoxical activity changes is not clear.

What are possible functions of the observed rate changes?

Sensory stimulation and initiation of motor activity accelerates heartbeating. In particular, activation of the neural program for swimming accelerates cycling of the heartbeat oscillator through central interactions. By accelerating the circulatory mechanism through a central interaction, the leech compensates for buildup of an oxygen debt and other concurrent metabolic needs resulting from muscular activity. *Hirudo* does possess some capacity for anaerobic metabolism, but under normal conditions takes up oxygen over its general body surface and depends on the circulation of hemoglobin-containing blood (Mann 1962; Zebe et al. 1981).

Activation of motor programs such as that for swimming also leads to excitation of Retzius cells and release of 5-HT in the CNS and periphery, ultimately raising blood levels of neurohumor (Willard 1981; Mason and Kristan 1982). At the periphery, serotonin modulates generation of muscular tension (Mason and Kristan 1982), while in CNS it enhances motor excitability and raises the likelihood of spontaneous episodes of swimming (Willard 1981). Coupling of rate increases in circulatory pumping with motor activation promotes mixing and more rapid distribution of neurohumors released at different sites.

What pathways mediate the rate changes?

Pathways that influence the cycling of the heartbeat oscillator are summarized in the flow diagram of Fig. 10. Activation of the motor program for swimming by stimulation of a central command element, cell 204, in isolated nerve cords leads to acceleration of heartbeat. Furthermore, whenever activity of longitudinal muscle motoneurons is initiated or increased by the CNS, whether or not such activity is recognizable as a particular motor program, there occurs an accompanying acceleration of heart rate. However, no motor neuron has been found that feeds back onto the timing oscillator. Some as yet unidentified neuron or neurons mediate the excitation of both body wall motor-

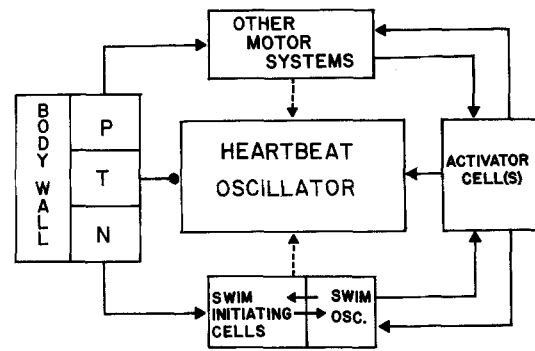


Fig. 10. Summary of pathways that affect the rate of heartbeat oscillator cycling. Arrows between boxes depict acceleratory (or excitatory) functional interactions. Lines ending in filled circles depict inhibitory interactions. See text for further explanation

neurons and the heartbeat system. The possibility of acceleratory pathways from the network that drives swimming and from other motor systems directly onto the heartbeat oscillator is indicated by dashed lines in Fig. 10. Mechanosensory neurons accelerate heartbeat oscillator cycling. This acceleration is brought about without the apparent production of psp's in HN cells. All acceleratory pathways that we have tested share this feature. Sensory cell activity is known to affect motor networks for shortening, local body movements and swimming (Nicholls and Purves 1970; Kristan 1982; Kristan et al. 1982; Weeks 1982c). It may be by acting on these systems that sensory neurons influence the heartbeat oscillator. Alternatively, mechanosensory cells may have independent, possibly direct pathways of access to activator neurons through which they affect heart rate. The influence of sensory cell activity on the heartbeat system long outlasts firing of individual sensory neurons. The rapidly adapting inhibitory pathway by which T cells influence HN cell firing (line ending in a filled circle, Fig. 10) appears not to affect DP activity.

Are any cells known that might have a function similar to the activator cells we have proposed? The Retzius cells exhibit properties that make them likely candidates for such a role. Retzius cell stimulation effects acceleration of heart rate accompanied by DP unit activity (Fig. 9). Initiation of swimming leads to activation of Retzius cells which themselves then feed back onto the swim motor system through the effects of the 5-HT they release in the CNS (Willard 1981). Bath application of 5-HT to isolated nerve cords at concentrations as low as 10^{-7} mol/l leads to acceleration of the heart rate (Arbas and Norris, unpublished observations). It remains to be shown, however, that 5-HT acts directly on HN cells of the timing oscillator.

By what mechanisms are the rate changes mediated?

The acceleratory pathways examined in this study mediate their long lasting effects without the apparent production of conventional psp's in HN cells. The rhythmicity of the heartbeat oscillator is the result of a fixed component – the pattern of connections among the HN cells plus several potentially variable components – including the efficacy of chemical synapses, and the endogenous oscillatory properties of HN cells. Modification of the latter class of properties in HN cells of the timing oscillator is the most likely path to prolonged acceleration of the heartbeat system.

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