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SWITCHING IN THE ACTIVITY STATE OF AN INTERNEURON THAT CONTROLS COORDINATION OF THE HEARTS IN THE MEDICINAL LEECH (*HIRUDO MEDICINALIS*)

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Summary

The rhythmically active heart interneuron HN(5) in the medicinal leech exhibits two distinct activity states, which have been associated with different coordination states of the two hearts. During the active state, it discharges high-frequency bursts of action potentials interrupted by rhythmic inhibitory input from other interneurons. In the inactive state, the same cell receives rhythmic inhibition but the membrane potential remains subthreshold between these volleys, producing few or no action potentials. We investigated differences in the membrane properties of the cell during the active and inactive states. The membrane potential in the active state oscillates on average between about −56±6 mV (S.D.) and −45±7 mV; the mean oscillation amplitude is 11±4 mV. In the inactive state, the membrane potential oscillates on average between −58±6 mV and −55±6 mV with a mean amplitude of 3±1 mV. The overall conductance of an HN(5) interneuron during the active state is approximately 10 nS lower than that during the inactive state, indicating that an outward current is turned off during the active state or turned on during the inactive state. This outward current is not voltage-dependent in the range −80 mV to −10 mV, as shown in voltage-clamp experiments by a linear current–voltage relationship. The reversal potential of this current is approximately −60 mV, indicating that chloride or potassium ions underlie the current. Using dynamic-clamp, we show that by adding an artificial current with a linear voltage-dependence (leak conductance) to an HN(5) interneuron (conductance 15 nS, reversal potential −60 mV), the cell can be transferred from its active to its inactive state.

Introduction

The circulatory system of the leech *Hirudo medicinalis* consists of two contractile lateral blood vessels (heart tubes) that extend longitudinally throughout the body. The heart muscle receives rhythmic excitation from segmental motor neurons, a pair of which is located in each of segmental ganglia 3–18 (Thompson and Stent, 1976*a*). The two contralateral heart motor neurons of the anterior ganglia burst alternately, while those in

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the rear ganglia burst in phase (Thompson and Stent, 1976*a*). The heart motor neurons on one side of the body produce bursts in a rear-to-front progression and cause a peristaltic wave of contraction in the heart tube on that side. The heart motor neurons of the other side burst more or less synchronously. The heart motor neurons of the two sides of the body switch their coordination mode simultaneously every 20–40 heartbeat cycles (Thompson and Stent, 1976*a*; Calabrese, 1977; Calabrese and Peterson, 1983). The peristaltic contraction mode in a lateral heart tube is associated with high systolic blood pressure; the synchronous contraction mode is associated with low systolic blood pressure (Krahl and Zerbst-Boroffka, 1983).

A pair of heart interneurons (HN interneurons) has been identified in each of the seven anteriormost segmental ganglia (Thompson and Stent, 1976*b*,*c*; Calabrese, 1977). The connections of a subset of these cells to each other and to heart motor neurons are drawn schematically in Fig. 1A. The HN interneurons of the first four ganglia are the oscillator interneurons; these form a network which is responsible for the timing of the heart beat (Peterson and Calabrese, 1982; Calabrese and DeSchutter, 1992). The heart interneurons HN(3), HN(4), HN(6) and HN(7) are synaptically connected to heart motor neurons,

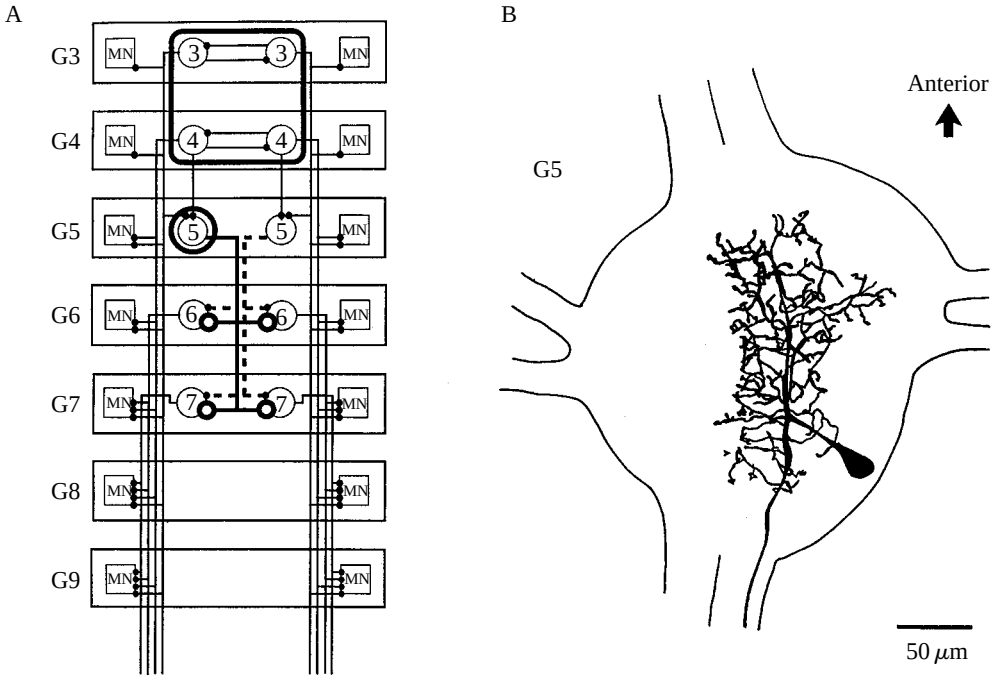

Fig. 1. (A) Diagram of the inhibitory synaptic connections among the heart interneurons HN(3)–HN(7) and the heart motor neurons (MN) in ganglia 3–9 (G3–G9). The ganglia are drawn as open squares. The heart interneurons HN(3) and HN(4) that produce the output of the timing oscillator are outlined in a box. The active heart interneuron (5) is encircled and its processes are drawn with solid lines, whereas those of the contralateral inactive heart interneuron (5) are drawn with dashed lines. (B) Structure of an HN(5) cell from the right side of the ganglion, ventral view. *Camera lucida* drawing from a cell stained with Lucifer Yellow.

whose activity they shape into rhythmic bursts (not all the HN interneurons are included in Fig. 1A).

The heart interneurons of the fifth ganglion [HN(5) cells] are not part of the timing oscillator and are not connected to heart motor neurons. The connection diagram in Fig. 1A shows that an HN(5) cell receives inhibitory input from ipsilateral oscillator interneurons HN(3) and HN(4). Thus, the two HN(5) interneurons are inhibited rhythmically in antiphase. Both the HN(5) interneurons make inhibitory synaptic connections to the ipsi- and contralateral heart interneurons of the sixth and seventh ganglia [HN(6), HN(7)].

At any given time, only one of the HN(5) cells is active, which means that it produces a burst of action potentials during the interval between the inhibitory postsynaptic potential (IPSP) volleys from the timing oscillator. Only this HN(5) interneuron effectively inhibits the HN(6) and HN(7) cells and thus transfers the rhythm of its (ipsilateral) side of the timing oscillator to the more posterior heart interneurons, which are synaptically connected to heart motor neurons. The inactive contralateral HN(5) cell produces few or no action potentials and does not inhibit its postsynaptic cells effectively (Calabrese, 1977). The result of the activity of all the heart interneurons is that the heart motor neurons on the side of the body ipsilateral to the inactive HN(5) interneuron are in the peristaltic coordination mode, whereas those ipsilateral to the active HN(5) cell are in the synchronous mode.

Every 20–40 heartbeat cycles, the two HN(5) cells switch their activity state, as shown by simultaneous recordings from the two contralateral HN(5) cells (Calabrese and Peterson, 1983). At the same time, the motor neurons switch their coordination mode. There is evidence that the HN(5) interneurons are crucial for determining the coordination mode of the heart beat, because intracellular current injection into an HN(5) neuron elicits a switch in coordination mode in the ipsilateral heart motor neurons (Calabrese, 1977).

The mechanisms by which the HN(5) interneurons switch their activity are unknown. A reciprocal switch cannot be induced by intracellular current injection into one HN(5) cell. We do not know whether the switch is caused by the intrinsic properties of the HN(5) interneurons or by external sources, such as input from other neurons. To address this problem, we have begun to investigate the membrane properties of the HN(5) cells.

Materials and methods

The leeches, *Hirudo medicinalis* L., obtained from Leeches USA and maintained in artificial pond water at 15 °C, were anesthetized in ice-cold saline and dissected. Usually an isolated chain of ganglia 4–6 was pinned out in a Sylgard (182, Dow Corning) lined dish, ventral side up. The fifth ganglion, and occasionally also the fourth, was desheathed with fine scissors and superfused with normal saline containing (in mmol l^{-1}): NaCl, 115; KCl, 4; $CaCl_2$, 1.8; glucose, 10; Tris buffer, 10; adjusted to pH 7.4 with HCl (Nicholls and Baylor, 1968); the flow rate was approximately 0.5 ml min^{-1} and the bath volume was approximately 0.2 ml. The experiments were performed at room temperature (approximately 23 °C).

To investigate the activity of interneurons HN(5) in the central nervous system (CNS), we used borosilicate glass microelectrodes (o.d. 1 mm, i.d. 0.75 mm) filled with 4 mol l^{-1} potassium actetate and 20 mmol l^{-1} KCl. When voltage- or current-clamp measurements were made, the tips of the electrodes were coated with Sylgard (186) to reduce the capacitance (resistance 30–40 MΩ). The microelectrodes were inserted intracellularly into the soma of the HN(5) cells and recordings were made using an Axoclamp amplifier (Axon Instruments) and displayed conventionally. The sampling rate during the measurements in discontinuous current-clamp (DCC) mode and discontinuous single-electrode voltage-clamp (dSEVC) mode was always above 4 kHz, the gain in dSEVC was 20–25 nA mV^{-1}, and the output bandwidth was 0.3 kHz at lower sampling rates or 1 kHz at higher sampling rates. An additional oscilloscope was used for monitoring electrode settling during the clamp experiments. The signals of the cells were amplified and stored on videotape. The voltage steps during dSEVC recording mode were programmed using Pclamp software (Axon Instruments), which also allows digitization (*via* the Labmaster interface) and analysis of the data.

HN(5) cells were identified by their rhythmic activity and the posterior-lateral location of their soma.

For the *camera lucida* drawing in Fig. 1B, the fluorescent dye Lucifer Yellow (Stewart, 1978) was injected intracellularly into an HN(5) cell using hyperpolarizing current (0.2 nA). The ganglion was then fixed in paraformaldehyde (4 %), cleared in glycerol (containing 0.15 % propyl gallate) and viewed with a fluorescence microscope.

Measurements are given as the mean value ± S.D.; *N* indicates the number of trials.

Computer software controlling the Axoclamp amplifier (Dynamic clamp, Sharp *et al.* 1993) was used to introduce an artificial leak conductance into an HN(5) cell. To apply dynamic-clamp, the recording was made in discontinuous current-clamp mode. The holding current injected into the cell was calculated according to the recorded membrane voltage, a specified reversal potential and a specified maximal conductance.

Results

The structure of an HN(5) interneuron in the fifth ganglion is shown in Fig. 1B. From the soma, which is situated ventrally in the posterior packet of neuronal somata, the neurite proceeds to the center of the ganglion, before looping back and leaving the ganglion through the connective to the sixth ganglion. The branches of the HN(5) cell are located ipsilateral to the soma in the anterior and posterior parts of the ganglion. The structure of the branches of the HN(5) interneuron in ganglia other than the fifth is not yet known (Shafer and Calabrese, 1981).

An HN(5) cell displays volleys of large inhibitory postsynaptic potentials, which are caused by the ipsilateral HN(3) and HN(4) neurons. The volleys last several seconds and shape the activity of the HN(5) cells (Figs 1A, 2). In our experiments, the mean period of the rhythmic activity was 14.6±4.7 s (*N*=10). For a given preparation, the period remained the same during the whole recording; however, there was considerable variation of period in different preparations.

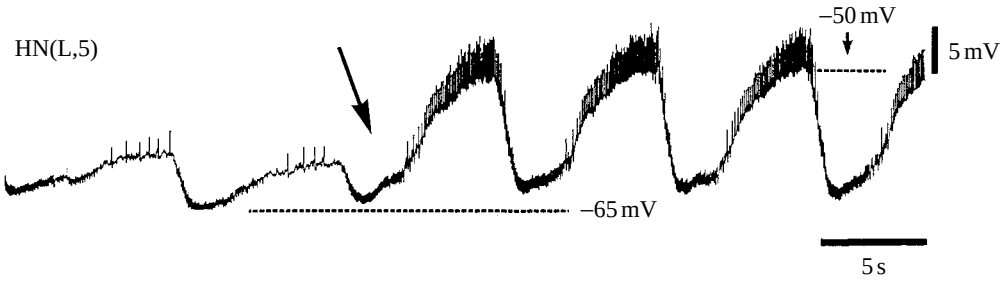

Fig. 2. Recording from a heart interneuron on the left side of the body [HN(L,5)] during a switch (large arrow) from the inactive to the active state. The membrane potentials are indicated by the dashed lines.

Description of the membrane potential in the active and the inactive state of the HN(5) cells

We have measured the membrane potential of the HN(5) interneuron, comparing the active and inactive states, to obtain a deeper insight into how the cell alternates between the two states over periods of several minutes.

During the inactive state, the membrane potential of the HN(5) cells oscillates between an average of −58±6 mV (S.D.) and −55±6 mV (Fig. 2); the average amplitude of the oscillation is 3±1 mV (*N*=10). In contrast, during the active state, the trough of the oscillation is on average −56±6 mV, the peak is −45±7 mV and the average amplitude is 11±4 mV (*N*=10). Large depolarizing potentials between volleys of IPSPs are characteristic of the active state. During the inactive state, the depolarizing phase does not show a rapid increase in membrane potential. The membrane potential measurements described above were performed in 10 different HN(5) cells. Because of the possibility of damage by the microelectrode, the actual membrane potentials may be more negative than those measured.

HN(5) cells typically switch spontaneously between the active and the inactive states. The mean number of cycles after which a switch occurred was 28±12 (*N*=9). For a given HN(5) cell, the number of cycles after which a switch occurred was fairly constant, differing by only a few cycles.

Usually, an activity switch in an HN(5) neuron occurred within one or one and a half heartbeat cycles (Figs 2, 3). Only in one HN(5) cell out of 21 different recordings did a switch from the active to the inactive state take several cycles. In 10 out of 20 recordings from different HN(5) cells, switches occurred during the volley of inhibitory potentials, resulting in the following cycle being fully expressed in the other activity state (Fig. 2). Switches occurred during the interval between the inhibitory potentials in 12 out of 20 different HN(5) cells (Fig. 3A,B). In Fig. 3A, a switch from the inactive to the active state is shown. The cell produces a small burst of action potentials which are terminated by the inhibitory inputs. By the next cycle, the neuron is in the fully active state. During a switch from the active state to the inactive state in the same cell (Fig. 3B), the membrane potential starts increasing, as in the active state, but only one action potential is produced before there is an abrupt decline in the potential.

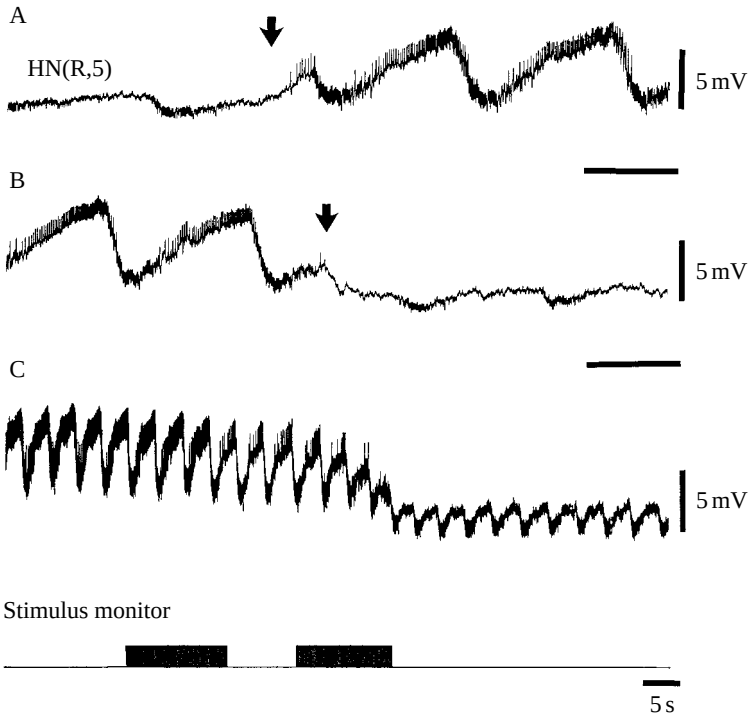

Fig. 3. Recording from an HN(R,5) cell during a spontaneous switch (arrow) from the inactive to the active state (A), during a spontaneous switch (arrow) from the active to the inactive state (B) and during a switch from the active to the inactive state induced by stimulating the contralateral connective between the fifth and sixth ganglia (C). The stimulus in C consisted of trains of pulses (duration 5 ms, amplitude 5 V, repetition rate approximately 3 Hz). Between the recordings shown in A and B, 10.25 min elapsed; between B and C, 13 min.

For most HN(5) cells, the time during a heartbeat cycle at which switches occurred, i.e. during the inhibition or during the depolarizing phase, was constant [in 17 out of 20 different HN(5) cells] regardless of whether the cell switched from the active to the inactive state or *vice versa*. The two contralateral HN(5) cells in one ganglion usually switched in a similar manner.

A switch can be artificially evoked, although not reliably, by stimulating the connectives between the fifth and sixth ganglia. Fig. 3C shows an example in which the connective contralateral to the recorded HN(5) cell was stimulated. The artificially evoked switch took several cycles, as can be seen by the decline in the frequency of action potentials over several cycles before the switch. Once the switch had occurred, the cell remained in the inactive state. It is likely that the stimulation induced the switch, because the number of cycles between switches was 42 and 46 before stimulation, whereas only 28 active cycles occurred before this switch. During a stimulation in the following active state, the HN(5) neuron switched after 26 cycles. After stimulation, the number of cycles between switches returned to 45 and 43.

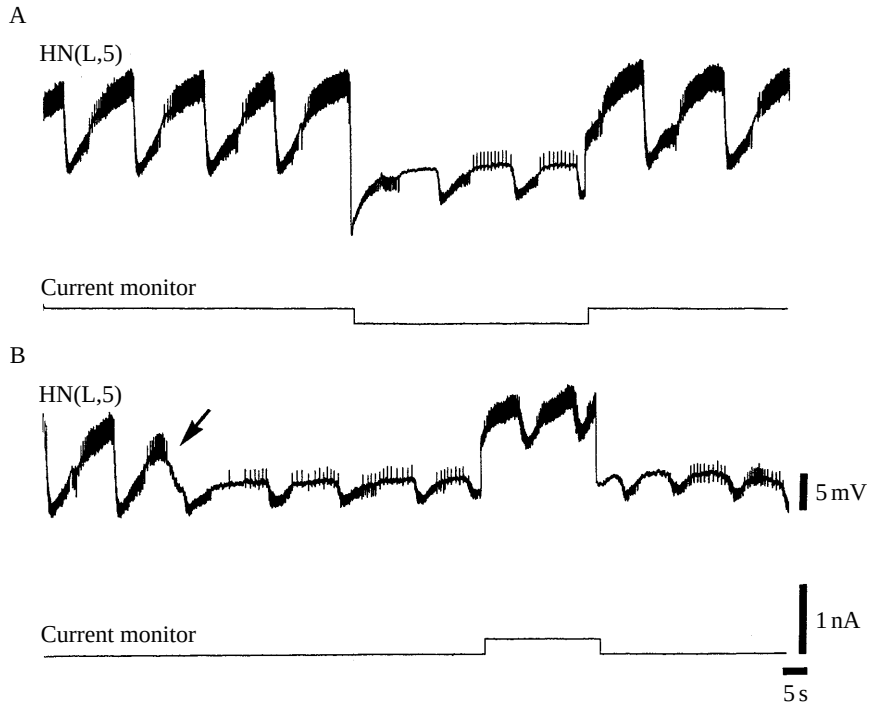

Fig. 4. Continuous recording from an HN(L,5) cell in discontinuous current-clamp mode. At the beginning, the cell is in the active state (A). The injection of a hyperpolarizing current (0.2 nA) elicits activity like that in the inactive state. The cell then switches spontaneously to the inactive state (B, arrow). A depolarizing current (0.2 nA) elicits activity like that in the active state.

Intracellular current injection can force an HN(5) interneuron temporarily into the other activity state. Fig. 4 shows, as an example, a recording from an HN(5) cell in discontinuous current-clamp mode. In the active state, the cell displayed activity like that in the inactive state when hyperpolarized by injection of 0.2 nA (Fig. 4A). The depolarizing potential was depressed by the current injection. After a spontaneous switch to the inactive state, the cell displayed activity like that in the active state when it was depolarized by 0.2 nA (Fig. 4B). In both cases, the cell returned to its previous activity state immediately after the intracellularly injected current was terminated.

Membrane properties in the active and inactive states of the HN(5) interneuron

Fig. 5 shows a recording from an HN(5) cell in the two activity states in which hyperpolarizing current pulses (−0.5 nA) were used to estimate input resistance. In discontinuous current-clamp recording mode, a train of pulses (duration 600 ms, rate 0.6 Hz) was delivered to the interneuron in the active state (Fig. 5, upper trace). After a spontaneous switch to the inactive state, another pulse train was delivered (Fig. 5, middle trace). The cell switched to the active state again and a third train of pulses was delivered (Fig. 5, lower trace). During the inactive state, the current pulses elicit smaller voltage

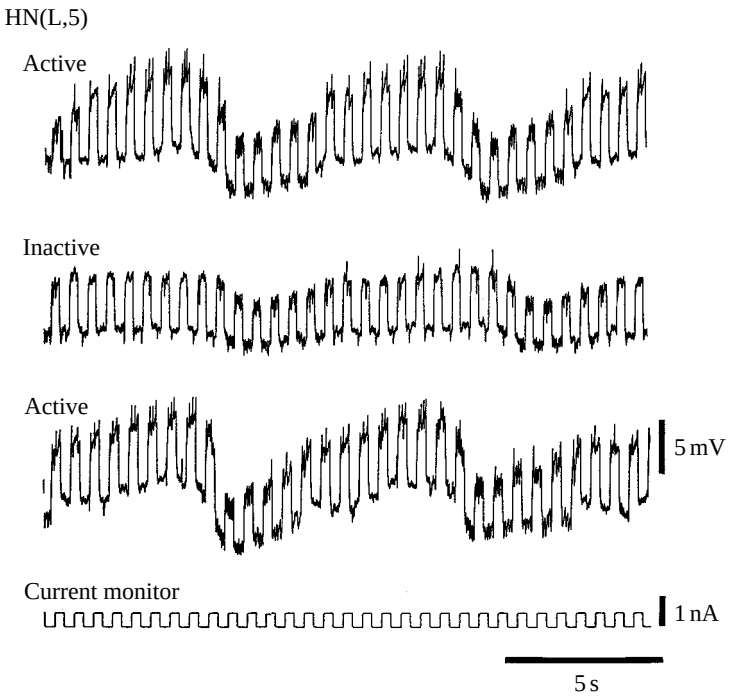

Fig. 5. Successive recording from an HN(L,5) interneuron in discontinuous current-clamp mode in the active state (upper trace), the inactive state (middle trace) and the active state (lower trace). For the measurement of the input resistance, hyperpolarizing pulses of −0.5 nA were applied (current monitor).

responses than they do in the active state, suggesting that the input resistance is lower in the inactive than in the active state.

To investigate the differences in input resistance revealed in the discontinuous current-clamp experiments (Fig. 5), we used the discontinuous single-electrode voltage-clamp technique. We used voltage ramps to measure the membrane conductance. Fig. 6 illustrates the experimental procedure. At the beginning of the example shown, the HN(5) interneuron was in the inactive state. For the indicated time, the recording mode was switched from bridge mode to discontinuous single-electrode voltage-clamp. A voltage ramp was then applied from the holding potential of −60 mV. After the ramp, the recording was returned to bridge mode to determine whether the cell was still in the inactive state. Four heartbeat cycles later, the cell switched spontaneously to the active state. A second voltage ramp was applied during the indicated time, after which the recording was returned to bridge mode. The current responses of the cell in the active and inactive states were then compared by plotting of the current–voltage relationships (Fig. 7). The voltage ramp covered membrane potentials from −80 to −20 mV. Above approximately −40 mV, voltage-gated outward currents were activated and the current–voltage relationship during the active and inactive states became non-linear. The difference current, however, was linear throughout the investigated voltage range and in

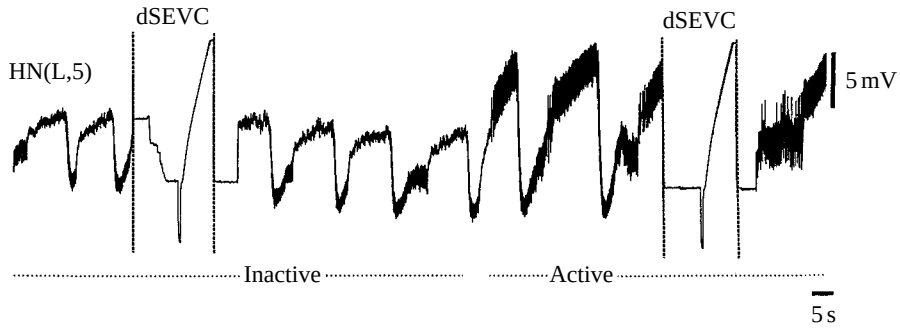

Fig. 6. Recordings from an HN(L,5) cell in the inactive and active states. During the times indicated, the recording mode was switched to discontinuous single-electrode voltage-clamp (dSEVC) and voltage ramps were applied.

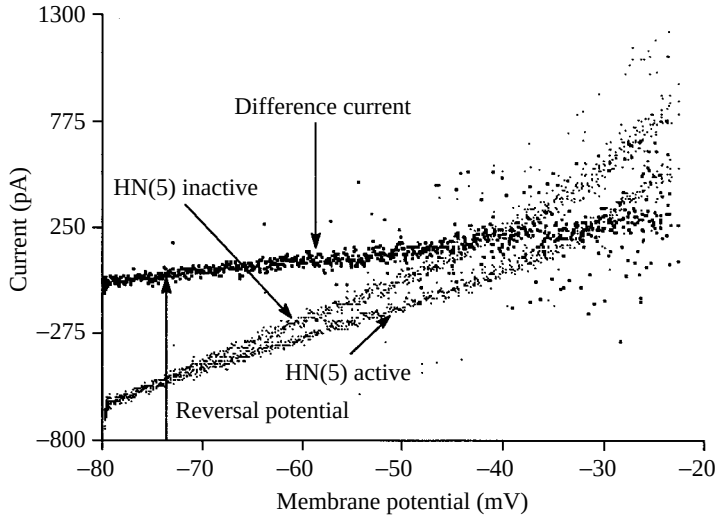

Fig. 7. Current–voltage relationship of an HN(5) cell in the active and the inactive states obtained in voltage-clamp experiments. The difference current is linear.

this case reversed at −74 mV. The overall conductance of an HN(5) cell in the active state is lower than that in the inactive state.

To quantify more accurately the conductance changes accompanying a switch and their reversal potential, a different voltage protocol from that shown in Fig. 7 was used. To avoid excessive spiking in the cells, a holding potential of −60 mV was usually used and a slow voltage ramp was applied; it lasted about 35 s and covered the range −60 mV to −40 mV. We used slowly rising ramps because we wanted to measure conductance over a period that covered more than one activity cycle. The current response of the cell was then digitized (Fig. 8). Only those parts of the current response during which inhibitory potentials had not occurred were used for further analysis, because during inhibition an

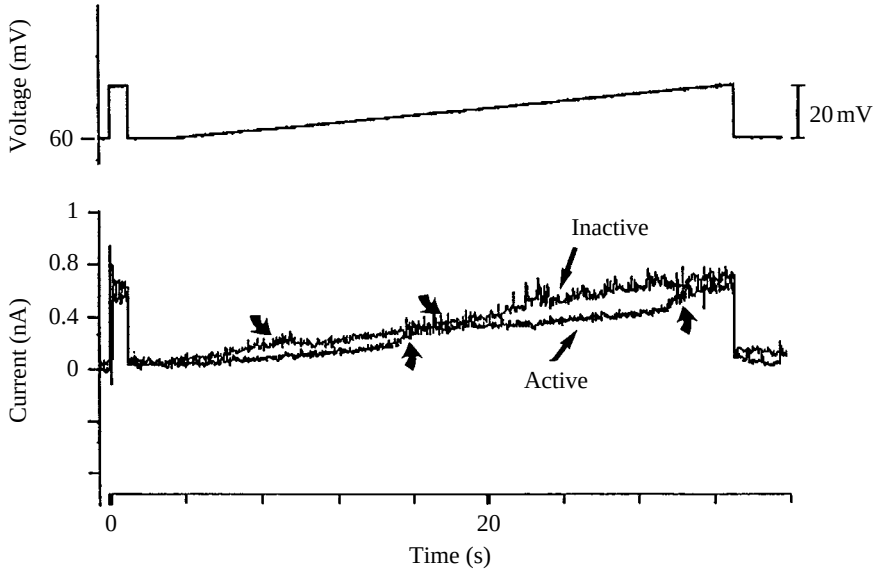

Fig. 8. Currents elicited in an HN(5) cell by voltage-clamp experiments in the active and the inactive states. A voltage ramp, rising approximately 35 s from −60 mV to −40 mV, was applied. The ramp covered several cycles of the heartbeat rhythm. The curved arrows point to the inhibitory synaptic currents during a heartbeat cycle.

additional conductance change is induced and linear regression could not be applied accurately. The current–voltage relationship was linear in the remaining parts of the response. The mean conductance of the evaluated difference current was 9.7±5 nS (*N*=8 different cells). It was calculated by subtracting the conductance in the active state from the conductance in the inactive state.

Fig. 9 illustrates an example in which conductances were calculated from the current response to the voltage ramp several times during consecutive active and inactive states of an HN(5) cell. The conductances in the two states differ in this case by an average of 13.5±3.5 nS (*N*=6) measured at the transition from the inactive to the active state.

The reversal potential of the linear difference current is −60.3±11.3 mV. It was determined as the point of the applied voltage ramp which elicited the same current response during both the active and the inactive states of a given cell, or, as in Fig. 7, as the voltage at which the difference current was zero. Table 1 illustrates the considerable variation in the measured reversal potentials. The value of the reversal potential suggests that chloride or potassium ions contribute to the linear current. Chloride currents have a reversal potential of −60 mV in the leech HN(3) and HN(4) neurons (Angstadt and Calabrese, 1991); potassium currents have a reversal potential of approximately −75 mV [Simon *et al.* 1992, measured in heart interneurons HN(3), HN(4) and HN(7)].

We used dynamic-clamp (Sharp *et al.* 1993) to add to an HN(5) cell an artificial conductance designed according to the parameters obtained from the experiments described above. Fig. 10 shows a recording from an HN(5) cell in discontinuous current-clamp mode. The cell was in its active state. At the indicated time, the dynamic-clamp

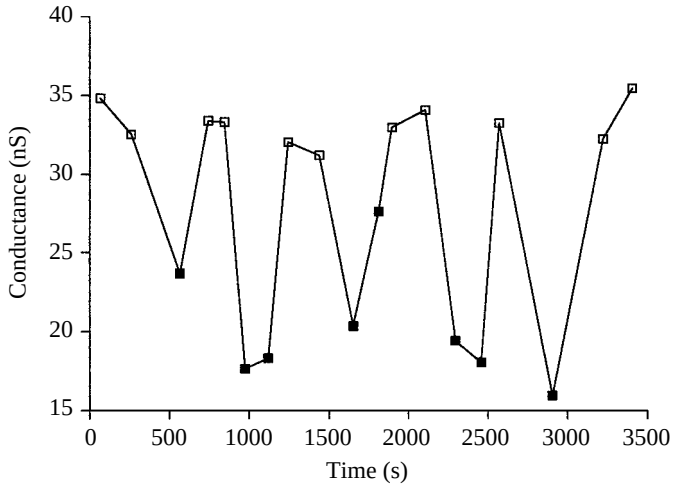

Fig. 9. Successive measurements of the membrane conductance in an HN(5) cell during several consecutive active (filled squares) and inactive (open squares) states.

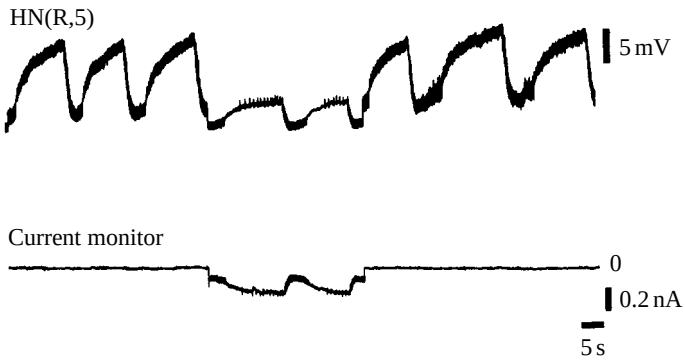

Fig. 10. Discontinuous current-clamp recording from an HN(R,5) interneuron during its active state. Dynamic-clamp was used to introduce into the cell an artificial conductance (current monitor), which was modulated according to the recorded membrane potential. During injection of this current, the cell displayed an activity like that recorded in the inactive state.

was switched on and a current, which was varied according to the membrane potential measured, was injected into the cell (lower trace). In the example shown, the reversal potential was specified as −60 mV and the conductance as 15 nS. Throughout current injection, the HN(5) cell assumed an activity like that in the inactive state. When released from dynamic-clamp, the cell resumed its active state.

Discussion

Our analyses of membrane potentials of HN(5) interneurons show that the active and

Table 1. *Reversal potentials of the difference current between the active and the inactive state measured in 10 different experiments*

Experiment number	Mean value of reversal potential (mV)	Number of measurements in one cell
1	–56.4	1
2	–60.4	1
3	–41.9	2
4	–55.6	1
5	–65.0	1
6	–63.3	3
7	–43.3	4
8	–68.1 (±4.7)	9
9	–75.4	1
10	–73.7	1
Mean value of reversal potential (*N*=10)	60.25 (±11.3)	

Values in parentheses are ± S.D.

inactive states are clearly distinguishable and that the transition between them is abrupt. The mean cycle period in our recordings is lower, and the average number of cycles between switches in the activity state is slightly higher, than previously reported (Calabrese, 1977; Peterson and Calabrese, 1982; Thompson and Stent, 1976*a*; Krahl and Zerbst-Boroffka, 1983). These differences may be due to the preparation used (a chain of ganglia 4–6), to the higher temperature at which our experiments were performed (15 °C *versus* 23 °C) or to changes in the ionic milieu within the ganglion, because in the experiments reported here, the outer capsule of the ganglion had been opened for better access to the neurons.

Our voltage-clamp data indicate that the difference between the membrane properties in the active and inactive states of HN(5) interneurons is a voltage-independent conductance. In the active state, the membrane conductance of the cell is lower and the cell is more depolarized than in the inactive state. The conductance differences and the reversal potential of the difference current between the active and the inactive states (−60 mV) indicate that the conductance is inhibitory and suggest that chloride ions or potassium ions contribute to it. In leech neurons, the reversal potential reported for chloride ions is −60 mV (Angstadt and Calabrese, 1991), −55 mV (Henderson, 1983) or −75 mV (Nicholls and Wallace, 1978); for potassium ions it is approximately −75 mV (estimated by Simon *et al.* 1992), −80 mV (Deitmer and Schlue, 1981) or −89 mV (Nicholls and Kuffler, 1964). It is also possible that the conductance is mixed. In this case, the reversal potential would be somewhere between the equilibrium potentials of the individual ions. Because the reversal potential of the current is relatively negative, however, it remains likely that potassium or chloride ions contribute to it. Because we have no means of artificially eliciting a switch in an HN(5) cell, it is not possible to test

the effect of a high intracellular concentration of chloride ions on the switching mechanism.

Our present interpretation of the function of this 'switching' current is that it tonically prevents the HN(5) cell from being active. When the current is turned off in the active state and the cell is relatively depolarized, voltage-gated currents in the cell could be activated to depolarize the cell further. This idea is supported by the observation that an inactive HN(5) cell, when depolarized, displays activity like that of a cell in the active state and *vice versa* (Fig. 4; Calabrese, 1977). When an artificial leak conductance was added to an active cell using dynamic-clamp, the cell became inactive as long as the dynamic-clamp was applied. This result shows that it is possible to change a cell from its active to its inactive state by introducing conductance that has no dependence on voltage. We do not know what turns this conductance on or off. The switch is probably not triggered by sensory input, because HN(5) cells switch regularly in isolated ganglia. The observation that an HN(5) cell changes its activity state only temporarily when depolarized or hyperpolarized indicates that switching is not due to inherent bistability. Stimulation of the connectives between the third and fourth ganglia, which do not contain the axons of HN(5) cells, can induce switches (Calabrese and Peterson, 1983), indicating that other neurons may be involved in inducing the switch.

Distinct neuronal activity states are expressed in many nervous systems, and in several of these cases a single identified neuron appears to play a key role in determining the different activity states. During gill ventilation in the crab *Carcinus maenas*, for example, the water flow can be directed forward or backward. A single interneuron (RSi1) has been identified in the thoracic ganglion that is depolarized by 15–20 mV during the spontaneously occurring reverse ventilation compared with forward ventilation. Stimulation of this neuron with depolarizing current during forward ventilation elicits reverse ventilation for the duration of the stimulus. Short stimuli can reset the forward ventilation rhythm. During reverse ventilation, brief hyperpolarization of the RSi1 cell ends this ventilation mode (DiCaprio, 1990). The mechanism which makes the neuron switch spontaneously between activity states is not known yet.

Several neurons of the stomatogastric nervous system of crustaceans are known to be switched between activity states by the action of neuromodulators (Dickinson and Moulins, 1992). One example is found in the lobster *Palinurus vulgaris*, where the neuron (VD), which is an integral member of the pyloric pattern generator, becomes a member of the cardiac sac pattern generator whenever the latter is active (Hooper and Moulins, 1989). The VD neuron switches from expressing the pyloric rhythm (rhythmic bursts at approximately 1 Hz) to expressing the cardiac sac rhythm (rhythmic bursts at approximately 0.05 Hz). During a burst in the slower cardiac sac rhythm, the cell is strongly excited. Between cardiac sac bursts, the cell is prevented from expressing the pyloric rhythm by the inactivation of its ability to generate plateau potentials (Hooper and Moulins, 1990).

Mammalian thalamic neurons display rhythmic oscillation in the absence of modulatory input, and tonic activity when modulatory inputs are active. The activity states are associated with sleep and wakefulness respectively. The amplitude of the modulatory input determines the level of depolarization by reducing the potassium leak

current, which, together with intrinsic properties of the cells, determines the activity state. A low-threshold calcium current (I_T) and a hyperpolarization-activated cation current (I_h), which promote the oscillation, can only be activated at more hyperpolarized membrane potentials (McCormick *et al.* 1992).

The HN(5) neurons of the leech seem to be similar to the RSi1 neuron of the crab in that the depolarization or hyperpolarization of a single neuron can alter the motor pattern. It is not known whether they also share intrinsic membrane properties. Our investigations show that the mechanism of the expression of the two activity states in an HN(5) neuron appears to be similar to that in thalamic neurons, in which the activity state is solely determined by the level of depolarization set by a voltage-insensitive conductance mechanism, and is unlike the neurons of the lobster stomatogastric nervous system, where switching results from modulation of voltage-gated currents. To clarify the mechanism of the activity switch in an HN(5) neuron, further investigations will focus on the current that underlies the depolarizing potential in the active state.

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