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Processing of sensory signals by a non-spiking neuron in the leech

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Abstract The non-spiking neurons 151 are present as bilateral pairs in each midbody ganglion of the leech nervous system and they are electrically coupled to several motoneurons. Intracellular recordings were used to investigate how these neurons process input from the mechanosensory P neurons in isolated ganglia. Induction of spike trains (15 Hz) in single P cells evoked responses that combined depolarizing and hyperpolarizing phases in cells 151. The phasic depolarizations, transmitted through spiking interneurons, reversed at around -20 mV. The hyperpolarization had two components, both reversing at around -65 mV, and which were inhibited by strychnine ($10 \mu\text{mol l}^{-1}$). The faster component was transmitted through spiking interneurons and the slower component through a direct P-151 interaction. Short trains (<400 ms) of P cell spikes (15 Hz) evoked the phasic depolarizations superimposed on the hyperpolarization, while long spike trains (>500 ms) produced a succession of depolarizations that masked the hyperpolarizing phase. The amplitude and duration of the hyperpolarization reached their maximum at the initial spikes in a train, while the depolarizations persisted throughout the duration of the stimulus train. Both phases of the response were relatively unaffected by the spike frequency (5–25 Hz). The non-spiking neurons 151 processed the sensory signals in the temporal rather than in the amplitude domain.

Key words Non-spiking · Leech · Sensory processing · Strychnine · Mechanosensory

Introduction

Processing of sensory signals into appropriate behavioral responses is accomplished at several levels throughout the neuronal network that connects sensory to effector neurons of the nervous system. The interneurons transmitting the signals are not mere conductors of the activity of a given sensory neuron, but they transform the input, extracting specific parameters of the environment (Burrows 1992). In consequence, the physiological properties of the interneurons are of utmost importance in the conversion of a sensory stimulus into an appropriate response.

In the nervous system, signals are codified and transmitted in two different electrophysiological modes: through (1) graded changes, and (2) all-or-none changes in the neuronal membrane potential. Neurons usually operate in both modes, processing the signal amplitude in a graded way up to a threshold beyond which, an all-or-none event takes place and the signal amplitude ceases to be an informative variable. Spikes encode information mainly in terms of the interspike period (Bullock 1993). Contrary to the spiking neurons, non-spiking neurons operate entirely – from input to output – in the graded mode (Burrows 1989). In this operation mode, neurons can encode information through amplitude and time (Manor 1997). Since their activity cannot be analyzed by means of extracellular recordings, non-spiking cells can only be studied in preparations where they can be identified and recorded using intracellular electrodes. Non-spiking neurons have been found in invertebrates playing the role of premotor interneurons (Pearson and Fourtner 1975; Burrows 1989; Büschges and Schmitz 1991) and acting as receptor neurons and interneurons in the vertebrate retina (Wässle and Boycott 1991).

In the leech, cells 151 are a pair of non-spiking neurons that can be clearly identified in each midbody

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ganglion along the nerve cord. These neurons are connected to several motoneurons by means of rectifying electrical synapses that allow a graded regulation of the firing frequency of the latter (Wadepuhl 1989). In addition, cells 151 are part of the interneuronal layer that mediates between sensory and motor neurons (Iscla et al. 1999). Given the wide influence of these neurons on effector neurons, it was of interest to analyze how sensory input impacts cells 151. Mechanosensory signals are a main drive of motor activity in the leech (Kristan 1982; Debski and Friesen 1987; Wittenberg and Kristan 1992), especially through the activation of the pressure-sensitive (P) neurons. Upon pressure exerted on the skin P cells fire a train of action potentials at approximately 2–20 Hz (Nicholls and Baylor 1968; Carlton and McVean 1995). Intracellular stimulation of the P cells at a rate of 10 Hz activated the motoneurons responsible for defensive reflexes such as local bending (Lockery and Kristan 1990) and shortening (Wittenberg and Kristan 1992; Shaw and Kristan 1995). Throughout this study, we have investigated the synaptic connectivity between the P neurons and cells 151, and the way in which these non-spiking neurons process the mechanosensory signal. The experimental results showed that stimulation of the mechanosensory neurons transmit depolarizing and hyperpolarizing signals onto cells 151 via a layer of interneurons. The depolarizing signals in cells 151 reflected the duration and frequency of the stimulus train while the hyperpolarization rather reflected the “on” of the mechanosensory signal.

Materials and methods

Biological preparation

Hirudo medicinalis, weighing 2–5 g, were obtained from a commercial supplier (Leeches USA, Westbury, N.Y.) and maintained at 15 °C in artificial pond water. The animals were not fed for at least 1 month prior to dissection. Individual ganglia from segments 7–15 were dissected out and pinned to Sylgard (Dow Corning), ventral side up, in a chamber under constant superfusion (50 $\mu\text{l s}^{-1}$) with saline solution at room temperature.

Solutions and materials

The saline solution had the following composition (in mmol l^{-1}): NaCl 115; KCl 4; CaCl_2 1.8; MgCl_2 1; TRIS maleate 4.6; TRIS base 5.4; and glucose 10; pH 7.4 (control solution). To block synaptic transmission a high $\text{Mg}^{2+}/\text{Ca}^{2+}$ solution containing 20 mmol l^{-1} MgCl_2 and 1 mmol l^{-1} CaCl_2 was used, and discrimination between mono- and polysynaptic connections between neurons was made using a solution containing 10 mmol l^{-1} MgCl_2 and 10 mmol l^{-1} CaCl_2 . The osmolarity was kept constant by reducing the NaCl concentration. Picrotoxin and strychnine (Sigma, St. Louis, Mo.) were dissolved in the saline solution and applied using the superfusion system.

Electrophysiological recordings

Neuronal activity was recorded using intracellular electrodes connected to an amplifier (Axoclamp 2B, Axon Instruments, Foster City, Calif.) operating in the current-clamp configuration. Micro-

electrodes were pulled from borosilicate capillary tubing (FHC, Brunswick, Me.), filled with a 3 mol l^{-1} potassium acetate solution, and had a resistance of 20–40 $\text{M}\Omega$. The recordings were digitized using a TL-1 DMA interface and acquired using Clampex protocols (pClamp 6, Axon Instruments) at sampling frequencies of 1–3 kHz. P cells were stimulated by trains of suprathreshold step pulses (2–4 nA, 5 ms) delivered through the intracellular electrode by a stimulator which was triggered by the acquisition software. The responses of neurons 151 were recorded while holding their membrane potential at different values by injecting d.c. current through a bridge-balanced electrode. The bridge balance was checked periodically during the experiments.

P and 151 neurons were recognized by their soma location and their electrophysiological properties (Muller et al. 1981). The identity of neuron 151 was further confirmed by injecting a d.c. current of –10 nA to –12 nA, which inhibits the spontaneous firing of the annulus erector motoneuron (Wadepuhl 1989).

Recordings were analyzed using a commercial software (Axograph 3.5, Axon Instruments). The synaptic responses were quantified by measuring the maximum amplitude of the depolarization and the hyperpolarization from the baseline, and the time-integral during a period of 1 s starting from the first action potential in the P cell (see inset in Fig. 1). To estimate the time integral the baseline before stimulation was equated to zero and, using

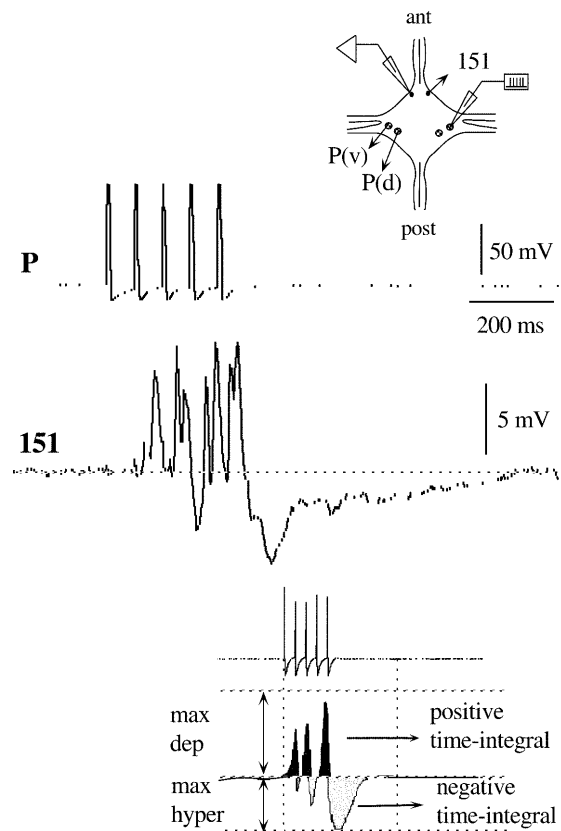


Fig. 1 Response of a neuron 151 to pressure-sensitive (P) cell stimulation. Recordings of the electrophysiological response of a cell 151 (*lower trace*) to stimulation of a P neuron (*upper trace*). Cell 151 was recorded at its resting potential and the P cell was stimulated to fire a train of five action potentials at 15 Hz. The *upper inset* represents the recording configuration: the isolated ganglion, showing the position of the pairs of ventral (v) and dorsal (d) P neurons and the pair of neurons 151. One microelectrode was used to stimulate the P cell and a second was used to record the response of the neuron 151. The *lower inset* shows the parameters used to quantify the response of the neuron 151 as described in Materials and methods

Axograph, the time integral was calculated as the algebraic sum of the membrane potential values during the defined period. Since the synaptic responses combined depolarizations and hyperpolarizations their quantification differentiated positive (dark gray) and negative (light gray) areas as indicated in the inset of Fig. 1. To carry on this analysis the traces were rectified (negative fragments in the trace were inverted) using Axograph, and the time integral in the original recording (x_o) and that of its rectified version (x_r) were measured. The negative area was calculated as $(x_o - x_r)/2$ and the positive area was calculated as $x_o - (x_o - x_r)/2$. Results are expressed as mean \pm standard error (SEM) and the number of neurons studied is given in parentheses (n). Curve fitting was achieved using a commercial software (Kaleidagraph 3.0.2 from Abelbeck software). Statistical significance of the results obtained in different experimental conditions was determined by t -tests.

Results

Responses of cells 151 to mechanosensory signals

The interaction between the mechanosensory P neurons and cells 151 was studied in isolated midbody ganglia using sharp electrodes inserted into the somata of these neurons. The activation of a train of action potentials in single P cells produced a complex response in cells 151, including both depolarizing and hyperpolarizing phases (Fig. 1). The depolarizing potentials exhibited rapid rising and falling time courses, they were not time-locked to the P cell spikes, and each spike could be followed by more than one depolarizing deflection.

To enhance the expression of the two components of the response, cells 151 were set at different membrane potentials by means of d.c. current injection in current-clamp configuration. Figure 2A shows representative results: at their resting potential, close to -40 mV, the train of action potentials in the P neuron caused depolarizing and hyperpolarizing deflections from the baseline. As the membrane potential of cell 151 was shifted to more positive values, the amplitude of the hyperpolarization increased. The hyperpolarizing phase had a relatively rapid onset but a very slow decay, and the positive deflections were superimposed on it. As the membrane potential was shifted to more negative values the amplitude of the hyperpolarizing component decreased and, conversely, that of the depolarizing deflections increased.

A close inspection of recordings ($n = 20$), such as those shown in Fig. 2A, revealed that, in most cases, the hyperpolarizing component reversed at around -70 mV. Since this is close to the equilibrium potential of chloride in leech neurons (McAdoo and Coggeshall 1976), inhibitors of receptors coupled to chloride channels were tested as a means to block the hyperpolarizing component. Picrotoxin (10 – $100 \mu\text{mol l}^{-1}$), an inhibitor of GABA receptors, had no effect on the response (data not shown; $n = 4$), but $10 \mu\text{mol l}^{-1}$ strychnine, an inhibitor of vertebrate glycine receptors, selectively blocked the hyperpolarization ($n = 4$). The recordings in Fig. 2B show that, in the presence of $10 \mu\text{mol l}^{-1}$ strychnine, the response of cell 151 at -40 mV was solely positive. The amplitude of the depolarizations increased as the membrane was shifted to

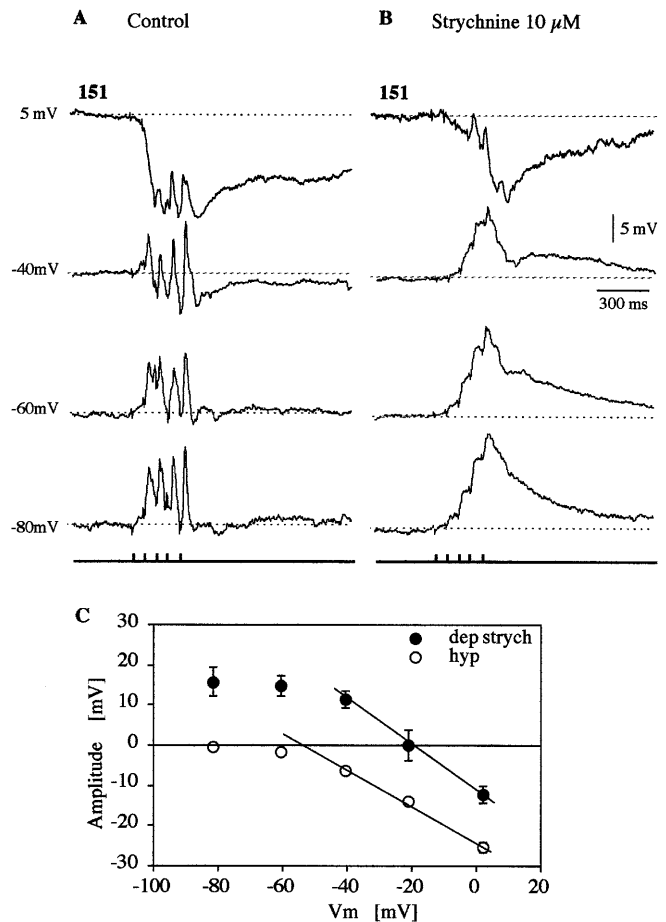


Fig. 2A–C Responses of a neuron 151 held at different membrane potentials. **A** The traces show the intracellular recordings of a neuron 151 as a P cell fired a train of five action potentials at 15 Hz. The dotted lines indicate the projection of the baseline of these recordings before the stimulus was applied. The responses of the cell 151 were recorded setting the cell at different membrane potentials, as indicated to the left of each trace. The vertical lines beneath the series of traces indicate the timing of the action potentials in the P cell. **B** Same as in **A**, in a solution containing $10 \mu\text{mol l}^{-1}$ strychnine. The traces in **A** and **B** were obtained from the same pair of neurons. **C** The graph shows the average maximum amplitudes of the depolarizing and the hyperpolarizing responses measured as a function of the membrane potential. The depolarizing responses were measured in recordings obtained in presence of strychnine (*dep strych*) and the hyperpolarizing responses were quantified from recordings obtained in control saline ($n = 4$). The bars indicate SEM

more negative potentials, and it decreased and reversed its sign as the membrane was shifted to more positive potentials. Washing out ($n = 3$) with control solution for approximately 20 min showed that the effect of strychnine was reversible (data not shown).

To estimate the reversal potential of the depolarizing response an analysis of the recordings obtained in the presence of $10 \mu\text{mol l}^{-1}$ strychnine was performed, and their maximum amplitude subsequently plotted as a function of the membrane potential (Fig. 2C). The plot fitted a line in the range 0 mV to -40 mV ($r = 0.99$) that indicated a reversal potential of -20 mV. Beyond -60 mV

the depolarization showed a noticeable rectification probably due to the anomalous rectification exhibited by these neurons (Wadepuhl 1989). The reversal potential of the hyperpolarization was estimated from recordings carried out in control solution. The peak hyperpolarization was plotted as a function of the membrane potential (Fig. 2C) and it showed a roughly linear relationship ($r = 0.99$) in the range -20 mV to -60 mV. Since the depolarization masked the reversed hyperpolarization the amplitude of the latter could not be measured beyond the reversal potential. The projection of the linear fit crossed the y -axis at -63.5 mV. The results suggest that P cell stimulation triggered two concomitant responses in the non-spiking neurons through the activation of different postsynaptic receptors.

Analysis of the receptive field of cells 151

The segmental ganglia contain two bilateral pairs of P cells. One pair innervates mainly the dorsal quadrants (Pd) and the other pair innervates mainly the ventral quadrants (Pv) of the skin (Nicholls and Baylor 1968). To assess whether neurons 151 discriminate among signals coming from different P cells, the maximum amplitude of the depolarization and the hyperpolarization of cells 151 triggered by P cell stimulation was measured at their resting potential. The results, summarized in Fig. 3, indicate that the depolarizing and hyperpolarizing responses activated by the four P cells showed, in average, no significant differences ($P > 0.1$). In conclusion, each cell 151 received similar inputs from P cells innervating dorsal and ventral aspects of ipsilateral and contralateral quadrants of the segmental body wall.

Synaptic connectivity of P and 151 neurons

The fact that the responses varied as a function of the membrane potential of the cells 151 indicates that the

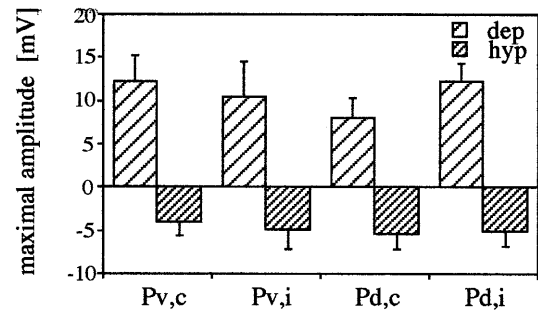
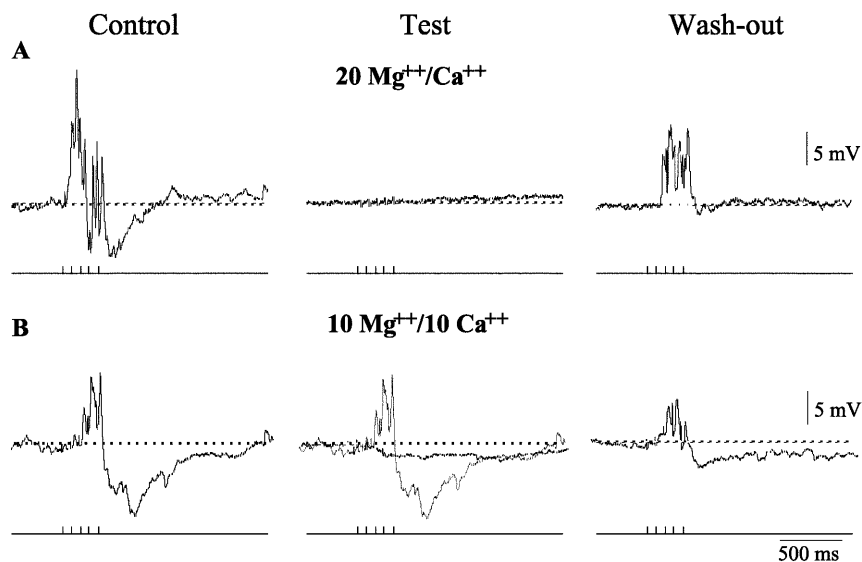


Fig. 3 Receptive fields of cells 151. Average maximum amplitudes of the depolarizing (*dep*) and hyperpolarizing (*hyp*) responses of cells 151, at their resting potential, to the four P cells in a ganglion ($n = 5$). P cells were stimulated with trains of five action potentials at 15 Hz. Ventral (*v*) and dorsal (*d*) P cells, ipsi- (*i*) and contralateral (*c*) to the recorded cell 151. The bars indicate SEM

signal from the P cell was transmitted through chemical junctions. To further examine whether the responses of neurons 151 to P cell stimulation were transmitted through chemical or electrical connections, the interaction of these neurons were put to the test in a solution containing a high Mg^{2+}/Ca^{2+} ratio (see Materials and methods). The two components of the 151 responses were completely nullified by this divalent cation ratio ($n = 3$), and washing out the ganglia with control saline solution showed that this effect was reversible (Fig. 4A). These results confirm that responses were transmitted through chemical synapses.

Fig. 4A, B Synaptic connectivity between cells 151 and P cells. Representative responses of a neuron 151 to P-cell stimulation studied successively in standard solution (*Control*), after 3 min superfusion with the test solution (*Test*), and after 10 min wash-out in standard solution (*Wash-out*). Cells 151 were held at their resting potential. The vertical lines beneath the traces indicate the timing of the action potentials in the P cell. In **A** the test solution contained a 20/1 Mg^{2+}/Ca^{2+} ratio. In **B** the test solution contained a 10/10 Mg^{2+}/Ca^{2+} ratio. In the latter case the control response was superimposed as a *gray trace* on the test response



To examine if the chemical responses were transmitted through mono- or polysynaptic pathways, experiments were carried out in a solution containing $10 \text{ mmol l}^{-1} \text{ Mg}^{2+}$ and $10 \text{ mmol l}^{-1} \text{ Ca}^{2+}$, which impairs polysynaptic pathways in the leech by shifting firing threshold to more positive values (Nicholls and Purves 1970). In this condition (Fig. 4B), the depolarizations were reversibly suppressed while the hyperpolarization was inhibited to $38 \pm 0.3\%$ of its control value ($n = 3$). As shown by the superposition of the traces obtained in control and test solution, the persistent response matched the late component of the hyperpolarization (Fig. 4B). This suggests that the high divalent cations removed the depolarizing phase and a shorter-lived component of the hyperpolarization, without affecting an underlying long-lasting component of the hyperpolarization.

Signal processing by cells 151

To examine how the expression of the depolarization and the hyperpolarization were influenced by the stimulus pattern, two features were varied: the number of action potentials at a constant frequency; and the frequency of a fixed number of action potentials.

To investigate the impact of the number of spikes, the response of cells 151 to trains of increasing number of action potentials were analyzed at a rate of 15 Hz. Short trains ($< 400 \text{ ms}$) of action potentials produced responses in which phasic depolarizations were super-

imposed on a slow hyperpolarization that outlasted the stimulus period; long trains ($> 500 \text{ ms}$), instead, produced only a succession of phasic depolarizations (Fig. 5). The hyperpolarizing phase was completely masked by the depolarizations generated by long stimulus trains, and these depolarizations markedly outlasted the stimulus period. These results also suggest that the hyperpolarization was maximally developed after the first few action potentials of a train, and it did not grow either in amplitude or in duration. To test this interpretation a comparison was drawn of the responses of cells 151 to short and long stimulus trains at two membrane potentials: (1) at around -20 mV , in which the hyperpolarization was the main component; and (2) at around -65 mV , a membrane potential in which the hyperpolarization was almost not expressed. Figure 6 shows representative results of these experiments. Superposition of traces depicting the responses to 3 spikes and 11 spikes, as cell 151 was held at -20 mV , shows that the temporal courses of the hyperpolarization produced by the two trains were very similar. On the other hand, superposition of the responses of the same cell, when it was held at -65 mV , coincided only during the period corresponding to the first 3 spikes. At the end of the response to the shorter train the cell went back to its basal level, while in response to the longer train the cell displayed a succession of depolarizations throughout the length of the stimulus.

To quantify the expression of each phase of the responses their maximum amplitude and also their time integral were assessed (see inset of Fig. 1) and the average values plotted as a function of the number of

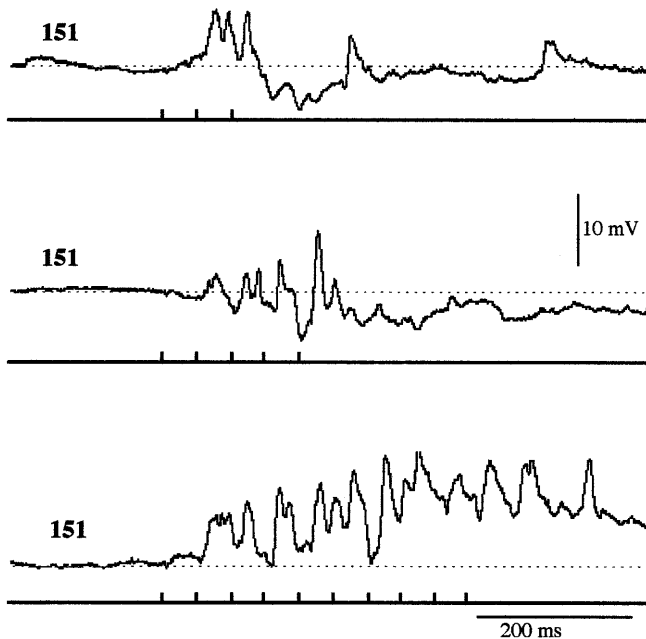


Fig. 5 Responses of a cell 151 to different spike numbers in the P cells. The three traces show the responses of a neuron 151 to three, five, and ten action potentials in a P neuron at 15 Hz. Cells 151 were at their resting potential and the vertical lines beneath the traces indicate the timing of the action potentials in the P cell

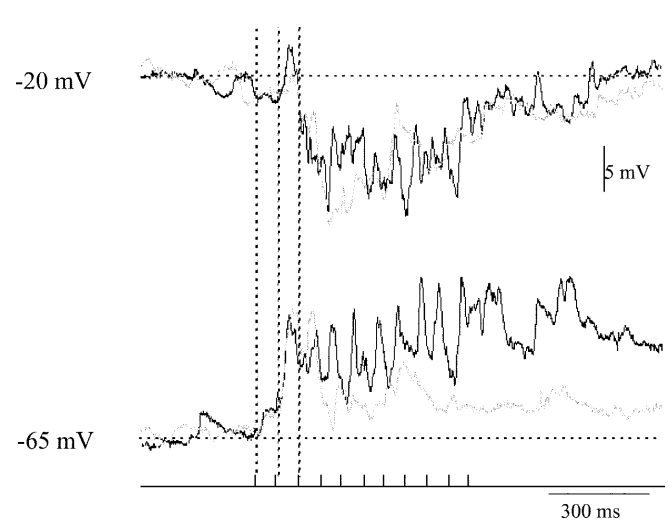


Fig. 6 Comparison of the time-course of the responses of a cell 151 to short and long trains of P cell stimulation. The traces show the responses of a 151 neuron to trains of 3 (gray) and 11 (black) action potentials at 15 Hz in a P cell, obtained when the neuron 151 was set at -20 mV and -65 mV (indicated on the left). The traces obtained at each membrane potential for the two stimulus patterns were superimposed; the vertical lines beneath the traces indicate the timing of the action potentials in the P cell. The vertical dotted lines indicate the timing of the first three action potentials in the P cell. Similar results were obtained in six other preparations

action potentials. In this way it was possible to evaluate to what extent the additional P cell spikes add to the magnitude and to the duration of the response. Figure 7 depicts the results of this assessment. The amplitude increased sharply with depolarizations produced by trains of two P cell spikes as compared to those produced by a single spike. The amplitude then remained at that level for additional spikes within short trains and experienced a further small increase in response to long trains. The positive area of the responses had quite a different behavior, increasing exponentially ($r=0.98$) upon successive spikes. This exponential relationship revealed that upon longer stimulus trains the depolarizing phase became more enduring, outlasting the stimulus period (Fig. 5). The hyperpolarization amplitude achieved an almost maximum level upon the first P cell spike; it remained at that level for up to three spikes; and, upon additional spikes, the hyperpolarization was no longer detectable. The negative area did not increase significantly even in the range of short trains, and it was nil with long trains.

To investigate the effect of the spike rate an analysis was carried out of the responses of cells 151 to trains of five action potentials at different frequencies (5–25 Hz). Figure 8 shows representative recordings in which trains at 5–25 Hz produced depolarizing responses that were

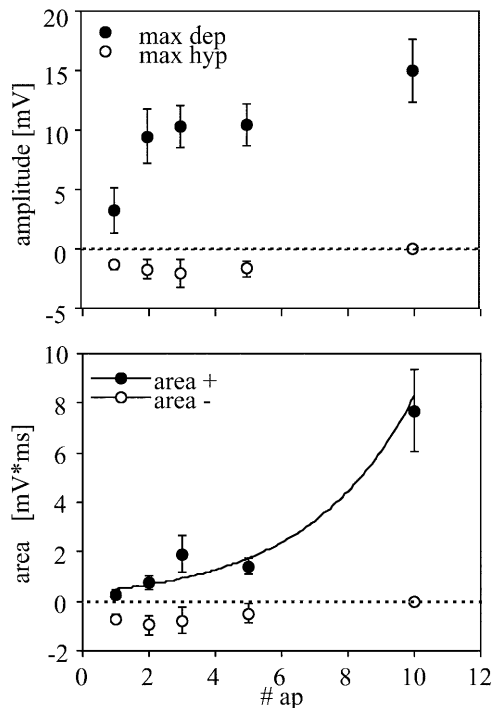


Fig. 7 Responses of cells 151 as a function of the number of P cell spikes. The upper graphs show the average maximum amplitude of the depolarizing and hyperpolarizing responses of cells 151 (see inset in Fig. 1) as a function of the number of P cell spikes. The lower graph shows the average positive and negative areas of the responses as a function of the number of P cell spikes. Both graphs were taken from the same set of recordings where cells 151 were set at their resting potential. The data for the positive area was fitted to an exponential curve. The bars indicate SEM ($n=5$)

superimposed on a prolonged hyperpolarization. The temporal distribution of depolarizations reflected the temporal course of the stimulus train but the hyperpolarization developed similarly at all the rates that have been studied. Figure 9 clarifies the temporal development of the hyperpolarization illustrating superimposed recordings of the responses of cells 151, held at -20 mV, to trains of 5 Hz and 25 Hz. The hyperpolarization in both recordings follows a very similar temporal course in spite of the different temporal characteristics of the stimuli triggering them.

Responses to stimuli at different frequencies were also quantified by measuring their maximum amplitude and their time integral (see inset of Fig. 1). Average values (obtained at resting potential) were plotted as a function of the spike frequency showing that the maximum amplitude of both phases and the positive and negative areas experienced no significant changes with the spike rate (Fig. 10).

Discussion

The present investigation shows that the non-spiking cells 151 received inputs from the mechanosensory P cells through multiple pathways. Spike trains fired by the P cells elicited a dual response of antagonistic action in cells 151. The hyperpolarizing response was formed by two phases: a briefer component transmitted through

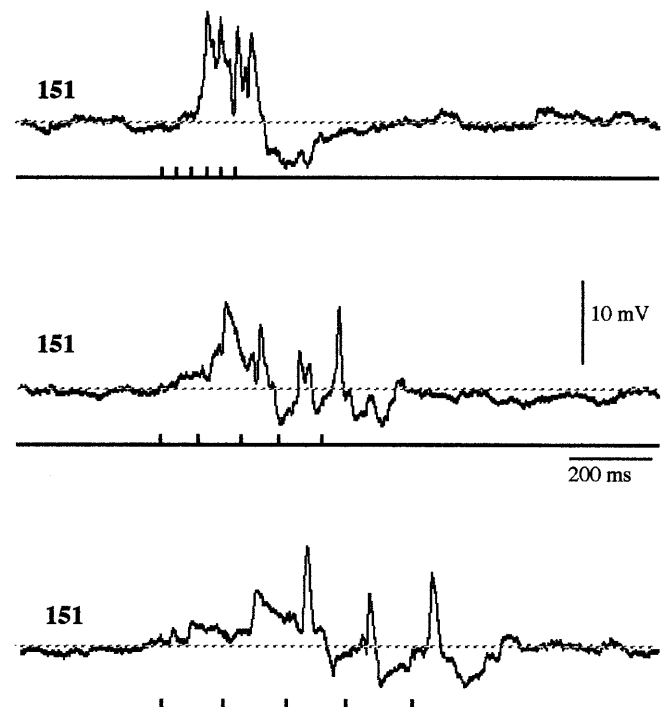


Fig. 8 Response of cells 151 to spike trains at different frequencies in the P cells. The three traces show the responses of a neuron 151 to five action potentials in a P neuron delivered at 25 Hz, 10 Hz, and 5 Hz. The vertical lines beneath each trace indicate the timing of the action potentials in the P cell

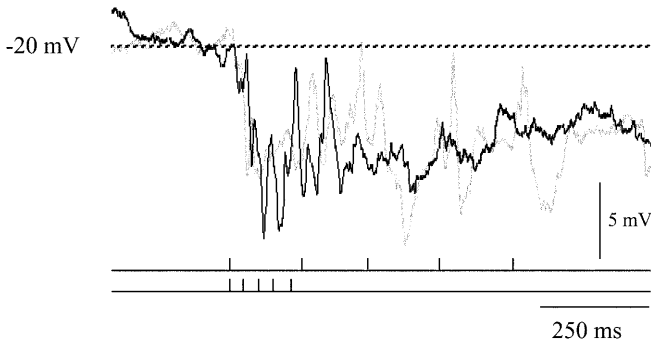


Fig. 9 Comparison of the time-course of the responses of a cell 151 to P cell spikes fired at 5 Hz and 25 Hz. The traces illustrate the response of a 151 neuron to trains of five P-cell spikes fired at 5 Hz (gray) and 25 Hz (black) obtained when the neuron 151 was set at -20 mV. The two traces were superimposed. The vertical lines beneath the traces indicate the timing of the action potentials in the P cell. Similar results were obtained in two other preparations

spiking interneurons, and a long-lasting component resulting from the direct action of the P neurons on cells 151. The depolarizations were seemingly conveyed through spiking interneurons. The observation that more than one depolarizing deflection could be evoked

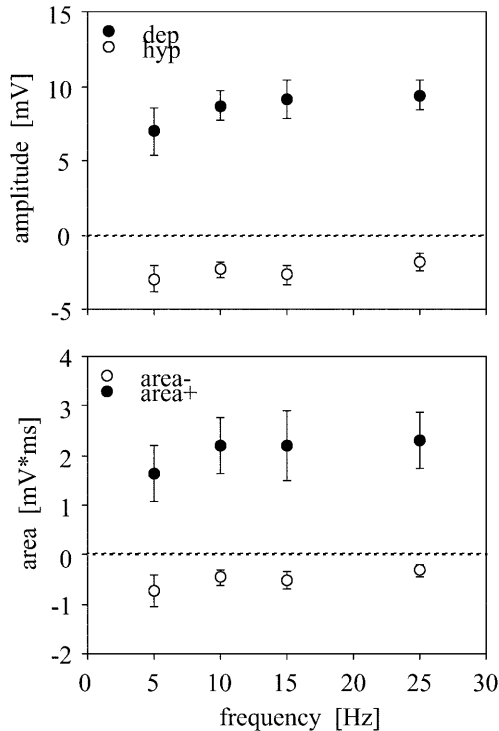


Fig. 10 Responses of cells 151 as a function of the P-cell spike frequencies. The upper graphs show the average maximum amplitude of the depolarizing and hyperpolarizing responses of cells 151 (see inset in Fig. 1) as a function of the P-cell spike frequencies. The lower graph illustrates the average positive and negative areas of the responses as a function of the P-cell spike frequencies. Both graphs were taken from the same set of recordings where cells 151 were set at their resting potential. The bars indicate SEM ($n=6$)

by a P cell spike suggests that the interneuronal layer was composed by more than one neuron acting in parallel or, alternatively, that each P cell spike could evoked a variable response in the interneuron. Therefore, in this reduced neuronal interaction between any of the four P neurons and cells 151 we observed the existence of several parallel pathways: at least one depolarizing interneuron, one hyperpolarizing neuron, and a direct action of P on 151 (Fig. 11).

It has been demonstrated that the spike trains of P cells evoked by pressure exerted on the skin lasted longer as this pressure increased beyond certain threshold (Carlton and McVean 1995). The P-151 interaction was highly sensitive to the train length and the depolarizing and the hyperpolarizing responses processed the number of spikes in different ways. The depolarizing deflections persisted throughout the duration of the stimulus train, in accordance with the timing of the P cell spikes. The hyperpolarization had a different time-course: it reached its maximum amplitude and duration within the first three action potentials of the stimulus train. Additional spikes did not extend the hyperpolarizing response, as if the interneuron(s) that transmitted this phase of the response only signaled the “on” of the mechanosensory signal, and were unresponsive to the duration of the stimulus.

These results show that two parallel pathways diverging from the same sensory neuron extracted different aspects from the same signal. The pathway transmitting the depolarization reacted to the time and the length of the sensory spike train, while the pathway transmitting the hyperpolarization encoded the onset of

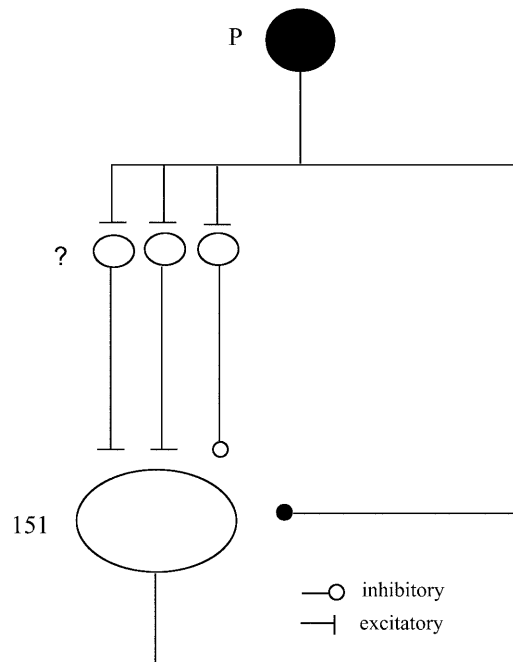


Fig. 11 Connectivity diagram describing the connectivity pattern between any of the four P cells and any of the two cells 151 in a segmental ganglion

the response ignoring the length or frequency of the sensory spike train.

As already mentioned, cell 151 was found to be electrically coupled to several motoneurons. Due to this connectivity pattern, the hyperpolarization of this neuron can exert a graded inhibitory effect on the spontaneous activity of the motoneurons (Wadepuhl 1989; Iscla et al. 1999). A possible role for an inhibitory "on" signal may be to reset activity in a group of motor neurons in preparation for a new motor response. The target of the depolarizing responses has yet to be identified. However, an ample correlation was found between the depolarizing deflections of cell 151 and the excitatory postsynaptic potentials displayed by the serotonergic Retzius neurons to the same P cell stimulation (Marín Burgin et al. 1999). These results suggest that cell 151 may project its depolarizing responses onto the serotonergic system of the leech.

Unlike spiking neurons, non-spiking neurons are able to encode information in terms of both amplitude and time. The responses of cell 151 to P cell stimulation showed that, in spite of its potential to translate information in terms of amplitude, this parameter displayed a very limited sensitivity to the spike frequency or spike number of the sensory signal.

The maximum amplitude of the depolarizing or hyperpolarizing responses was similar at spike frequencies between 5 and 25 Hz, a range within which the nervous system of the leech is behaviorally sensitive (Wittenberg and Kristan 1992).

The spike counts influenced the amplitude of the depolarizing responses mainly between the first and the second P-cell spike. The average amplitude of the depolarizing signal increased (paired *t*-test, $P < 0.05$) around threefold (Fig. 7) and maintained this value in response to additional P-cell spikes. With long spike trains the depolarization revealed an additional increase in amplitude of around 50% (paired *t*-test, $P < 0.05$), probably because the hyperpolarization ceased to develop. Instead, the most responsive parameter measured was the positive area that grew exponentially as a function of the number of P-cell spikes. The area measures the permanence of the depolarizing signal over time, and its exponential growth, in the absence of an amplitude increment, indicates that upon an increasing number of P-cell spikes the depolarization became more enduring.

Taken together these results suggest that cells 151 did not take advantage of their potentiality to develop gradual changes in membrane potential (Wadepuhl 1989) in handling the information from P cell neurons. Instead, the timing of the peaks and the overall period of sustained depolarization reflected the spike counts in the sensory neuron. This indicates that the temporal code was the prevalent information variable for this non-spiking neuron.

In this report we describe the interaction between a specific sensory neuron and a specific non-spiking neuron. Leeches are suitable for this kind of analysis due to the relatively low number of ganglionic neurons, their

almost invariable location and the large size of the soma. As mentioned before, the non-spiking cell 151, as non-spiking neurons described in the locust (Burrows 1980), is a premotor interneuron with influence on the activity of a variety of motor neurons. In spite of their similar role non-spiking neurons in the two species shows an interesting difference. The effect of non-spiking neurons of the locust on the motoneurons is exerted through graded chemical synapses (Burrows and Siegler 1978), while in the leech the signal is transmitted through rectifying electrical junctions (Wadepuhl 1989).

Populations of non-spiking neurons in the locust have been studied and classified in terms of their response to proprioceptors (Burrows et al. 1988) and exteroceptors (Laurent and Burrows 1988), and according to their distinct receptive fields. No other non-spiking neuron has been identified yet in the leech nervous system. Within the region of the ganglion where cell 151 is located all the other cells fire action potentials. The receptive field of the non-spiking cell 151 covers the four quadrants in which the segmental body wall of the leech is divided, which shows no field specialization.

The depolarizing responses of the locust non-spiking interneurons to sensory stimuli results from a monosynaptic transmission, while the hyperpolarizing responses proved to be disynaptic (Burrows et al. 1988). Both types of response took place simultaneously in cells 151 of the leech and both were transmitted through polysynaptic connections (with the exception of the slow hyperpolarization).

This general comparison suggests that cell 151 of the leech is located in a similar site within sensorimotor networks to the non-spiking interneurons of the locust. In both species they play the role of premotor neurons capable of exerting a gradual effect on the activity of motoneurons and they receive a complex sensory input. The leech non-spiking cell could be considered a simpler evolutionary version of the insect non-spiking cell population. The investigation of its role in the process of sensorimotor integration represents a simpler scenario in the investigation on how non-spiking neurons process sensory signals and contribute to the adjustment of movements.

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