ORIGINAL PAPER

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Network interactions among sensory neurons in the leech

Abstract Interactions among mechanosensory neurons, sensitive to touch, pressure and nociceptive stimuli in the leech nervous system were studied in isolated ganglia and in body-wall preparations. Pairs of touch-pressure, touch-nociceptive and pressure-nociceptive neurons were tested by suprathreshold stimulation of one neuron while recording the response of the other, in both directions. Pressure and nociceptive stimulation evoked depolarizing and hyperpolarizing responses in touch cells, mediated by interneurons. The relative expression of these responses depended on the stimulus duration. One or two pressure cell spikes produced, predominantly, a depolarization of the touch cells, and increasing number of spikes evoked a hyperpolarization. Nociceptive cells produced primarily the hyperpolarization of touch cells at any stimulus duration. When touch cells were induced to fire by injection of positive current into the soma, stimulation of pressure cells inhibited touch cell activity. However, when touch cells were induced to fire by peripheral stimulation, pressure cell activation failed to inhibit touch cell firing. The results suggest that excitation of pressure and nociceptive cells would not limit the responses of touch cells to peripheral stimuli, but would inhibit the firing of touch cells evoked by their central connectivity network.

Keywords Distal inhibition · Intersensory · Peripheral stimulation · Primary sensory neurons · Sensory network

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Abbreviations
N nociceptive · *P* pressure · *T* touch

Introduction

Sensory information takes place through multiple parallel pathways transmitting information vertically, from primary sensory receptors to higher processing levels; and laterally, through phenomena like recurrent and lateral inhibition, that refine the information extracted from external stimuli (Arbib et al. 1998; Boycott and Wässle 1999). Lateral interactions have been identified even at the level of primary sensory neurons: experiments performed in the retina of the goldfish show that besides the feedforward pathway between cones and horizontal neurons, a feedback synapse from horizontal cells to cones also exist (Verweij et al. 1996); in the mollusk *Hermissenda*, synapses between hair cells and photoreceptors have been described (Alkon 1973); and mechanosensory neurons of the leech display a wide set of interactions (Baylor and Nicholls 1969). A question that requires further attention is how the interactions among sensory neurons affect the processing of sensory information.

In the leech, mechanical stimulation onto the skin constitutes the main sensory drive to the major motor behaviors displayed by this annelid (Kristan 1982; Debski and Friesen 1987; Wittenberg and Kristan 1992). Deflections of the skin are detected by three main types of mechanosensory neurons that are sensitive to light touch (T), pressure (P) and nociceptive (N) stimuli. The nervous system of the leech reflects the simplicity of the architecture of the organism, composed of 21 midbody segments that display a highly similar structure, laying between two specialized head and a tail segments (Stent et al. 1992). Each midbody segment is innervated by a midbody ganglion containing the neuronal elements responsible for its sensory and motor functions. Thus, each ganglion contains a set of T, P and N neurons

innervating defined regions in the body wall of the corresponding segment.

Experiments performed by Baylor and Nicholls (1969) have established that the mechanosensory T cells form a network that links the whole set of T cells in each ganglion through chemical and rectifying electrical junctions, enabling the transmission of recurrent excitation and inhibition among homologous sensory neurons. Furthermore, these authors found that T cells receive a dual synaptic signal from P cells. This intersensory interaction opened the possibility that the responsiveness of T cells to mechanical stimuli could be tuned by the activity of the other mechanosensory cells. In the present study we have further investigated the interactions between the sensory neurons of different submodalities and the impact of the cross-connections in the electrophysiological activity of the sensory neurons. The results suggest that the mechanosensory neurons of higher sensory threshold (P and N cells) exerted an inhibitory action on the sensory neurons of lower threshold (T). This inhibitory signal impinged the T cells at a site electrically distant from the spike initiation zone activated by peripheral excitation of these neurons, but instead, effectively inhibited the spiking initiated centrally. The results suggest that excitation of P and N cells would not limit the responses of T cells to peripheral stimuli, but would inhibit the firing of T cells evoked by their central connectivity network.

Materials and methods

Biological preparation

Hirudo medicinalis, weighing 2–5g, 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 1month prior to dissection.

Two types of preparations have been used: isolated midbody ganglia and body-wall preparations. For the first type, individual ganglia were dissected out and pinned, ventral side up, in Sylgard-lined Petri dishes (Dow Corning), filled with saline solution at room temperature. The body-wall preparations consisted of a fragment of the skin, cut along its dorsal line, comprising around five segments, of which only the central segment was left innervated by the corresponding segmental ganglion. The skin was pinned down on the Sylgard basis of a dish and the ventral aspect of the ganglion was exposed by making a small hole in the skin. To ease the stretching of the body wall in the chamber, this maneuver was performed in a solution containing $10mmoll^{-1}$ Mg^{2+} and $1mmoll^{-1}$ Ca^{2+}, that abolishes chemical transmission and minimizes muscle contractions. Thereafter, the solution in the chamber was exchanged with normal saline, washing out the tissue with 5 times the volume of the dish, to assure the effective wash out of the high Mg^{2+} concentration. The sheath covering the ganglion was removed in both preparations.

Solutions and materials

The saline solution had the following composition (in $mmoll^{-1}$): 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. To block chemical synaptic transmission we used a solution containing $10mmoll^{-1}$ $MgCl_2$ and $1mmoll^{-1}$ $CaCl_2$, and to discriminate between mono- and polysynaptic connections among neurons we used a solution containing $10mmoll^{-1}$ $MgCl_2$ and $10mmoll^{-1}$ $CaCl_2$ (Nicholls and Purves 1970). In both cases the osmolarity was kept constant by reducing the NACl concentration.

Electrophysiological recordings

The neuronal activity was studied by means of intracellular electrodes impaled in the soma of identified neurons. T, P, and N neurons were recognized by their soma location and their electrophysiological properties (Muller et al. 1981).

The microelectrodes were pulled from borosilicate capillary tubing (FHC, Brunswick, Me., USA), filled with a $3moll^{-1}$ potassium acetate solution, and had a resistance of 20–40MΩ. The electrodes were connected to an amplifier Axoclamp 2B (Axon Instruments, Union City, Calif., USA) operating in the current-clamp configuration, and were bridge-balanced. The recordings were digitized using a Digidata 1320 interface and acquired using Clampex protocols (pClamp 8.0.2, Axon Instruments) at sampling frequencies of 10kHz. P and N cells were stimulated by trains of suprathreshold step pulses (2–4nA, 5ms) delivered through the intracellular electrode by a stimulator Master 8 (A.M.P.I., Jerusalem), which was triggered by the acquisition software.

In the body-wall preparations the mechanosensory neurons were recorded as the skin was stimulated mechanically by the application of pressure pulses of controlled magnitude and duration. These stimuli were delivered through a micropipette, which had a tip of around 5 öΥm, by a pressure-pulse generator Picospritzer II (General Valve, Fairfield, N.J., USA). These pressure pulses resulted in the localized bubbling of the external solution. The tip of the micropipette was directed to specific regions of the skin with the aid of a micromanipulator.

Recordings were analyzed using Axograph 4.5 (Axon Instruments). The synaptic responses were quantified by measuring the maximum peak amplitude of the responses from the baseline, or the resulting change in T cell firing. Results are expressed as mean ± SEM and the number of independent observations is expressed between brackets (*n*). Statistical significance of the results obtained in different experimental conditions was determined by *t*-tests.

Results

Stimulation of P and N cells affected the electrophysiological activity of T cells

The synaptic interactions between mechanosensory T, P and N cells were studied in isolated midbody ganglia, using intracellular electrodes, through which the neurons could be recorded and stimulated. Pairs of T-P, T-N and P-N neurons were tested by injecting suprathreshold current pulses in one neuron while recording the response of the other, in both directions. In agreement with previous studies (Baylor and Nicholls 1969) it was found that stimulation of T cells did not cause any response in P ($n = 5$) or N ($n = 4$) neurons and we did not record any interaction between N and P cells ($n = 4$). But, stimulation of P or N cells caused dual responses in T cells. Trains of action potentials in P or N elicited excitatory and inhibitory changes in the membrane potential of T cells, that generated a family of response types. Figure 1 shows representative recording of two different types of responses observed. The hyperpolar-

ization was the most conspicuous response for both pairs, and it took place as the sole response or in combination with an initial depolarization (Fig. 1A). Also, because T cells exhibit postinhibitory rebound excitation, action potentials could be elicited at the end of the hyperpolarization, generating responses like the one shown in Fig. 1B. In a few cases, T cells responded with a sole depolarization.

Overall, we distinguished four different response shapes and Fig. 1C and D summarize their occurrence for P-T and N-T interactions, respectively. The most marked difference between the response profiles evoked by P and N cells resides in that the depolarizations were evoked more frequently by P than by N. The sole depolarization of T cells was equally rare when both P or N were stimulated, but the hyperpolarizing responses evoked by P cells were frequently accompanied by depolarizations, and only around 25% of the responses were a sole hyperpolarization. However, N cell stimulation produced a sole hyperpolarization in around 45% of the cases.

The segmental ganglia contain two bilateral pairs of P cells and of N cells. The lateral P cells in the ganglion innervate the ventral quadrants of the skin, whereas the medial P cells innervate the dorsal quadrants (Nicholls and Baylor 1968). The pair of N cells innervate similar areas, extending from the dorsal midline to the ventral midline (Blackshaw et al. 1982). Although in a given ganglion different P or N neurons produced different responses, no pattern could be observed upon the activation of particular P or N cells throughout different preparations (data not shown).
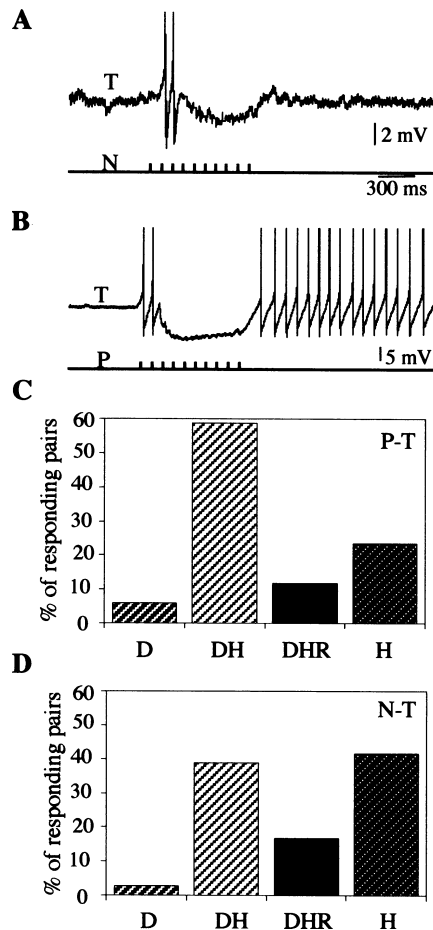
Signal processing by T cells

On examining whether the expression of the depolarizing and hyperpolarizing phases of the T cell responses depended on the properties of the stimuli applied in P or N, we found that the stimulus duration had a noticeable influence. The responses of T cells to trains of increasing number of action potentials in P or N neurons were analyzed. Figure 2 shows representative examples of the responses of T cells to two, five and ten action potentials in P (Fig. 2A) and N (Fig. 2B) cells, at a rate of 15Hz. The most prominent result was that increasing the number of spikes in P or N cells produced an increase in the amplitude and duration of the hyperpolarization. The average amplitude of the hyperpolarization increased with successive spikes in P and N (Fig. 2C). No significant differences were found between the responses produced by P or N cells at any stimulus length ($P > 0.05$, t-test). As already shown, the T cell responses included an initial excitation, which was more often produced by P than by N stimuli (Fig. 1C and D). The examples in Fig. 2A show that this excitation predominated in response to short stimulus trains and was curtailed by the increasing weight of the hyperpolarization produced by additional P cell spikes. Figure 3 describes the percentage of T cells that did not respond (none), responded with a depolarization, a depolarization followed by an hyperpolarization or an hyperpolarization, when different numbers of spikes were evoked in P and N cells. The characteristic profile of responses evoked by P and N cells (Fig. 1C and D) was further expressed in this study. As the duration of the P cell stimulus increased, the responses shifted from a predominant depolarization to the combination of depolarizing and hyperpolarizing phases. In contrast, N cell spikes hardly evoked any sole T cell depolarization, and increasing the spike number evoked sole hyperpolarizations or the combination of depolarizing and hyperpolarizing phases.
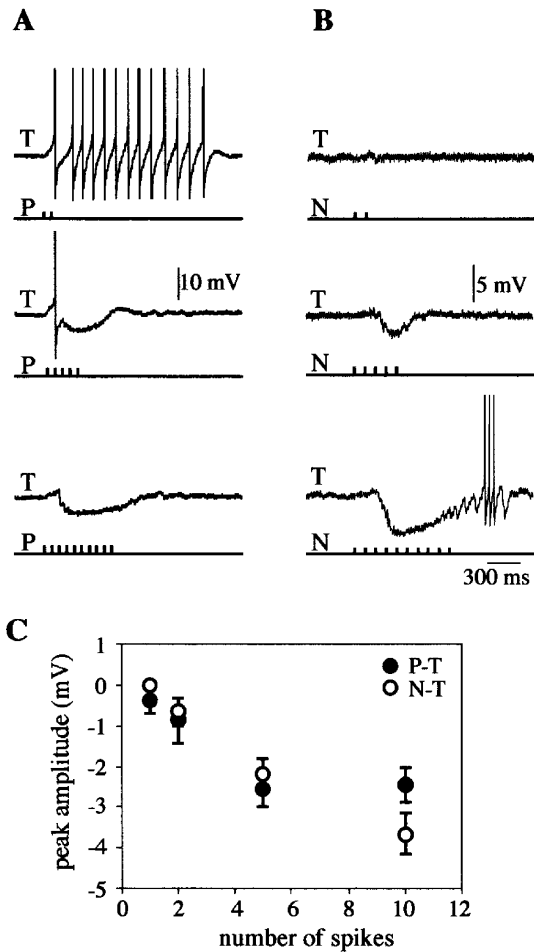

Fig. 1A–D Responses of touch (T) cells to intracellular stimulation of pressure (P) or nociceptive (N) cells. Intracellular recordings of T cell responses to stimulation of N (**A**) and P (**B**) neurons. T neurons were recorded at their resting potential and P or N cells were stimulated with a train of suprathreshold stimuli that elicited a train of ten action potentials at 15Hz. The *vertical lines* beneath the traces indicate the timing of the action potentials in the P or N cells. **C, D** The columns indicate the percentage of T cells that responded solely with a depolarization (**D**); a depolarization followed by a hyperpolarization (*DH*, as in **A**), a depolarization followed by a hyperpolarization, followed by a rebound (*DHR*, as in **B**); or only a hyperpolarization (**H**). **C** Responses of 25 different P-T pairs; **D** responses of 27 N-T pairs

Fig. 2A–C Responses of T cells to spike trains of different duration. The traces show the responses of a T neuron to two, five and ten action potentials in P (**A**) and N (**B**) cells, at 15Hz. The *vertical lines beneath the traces* indicate the timing of the action potentials evoked in P or N. **C** The graph shows the average peak amplitude of the hyperpolarization induced in T cells as a function of the numbers of spikes evoked in P (*n* = 11) and N (*n* = 16 pairs)

Fig. 3 Profile of T-cell responses as a function of the number of spikes evoked in P or N. The *bars* in each graph indicate the prcentage of P-T (*n* = 11) and N-T (*n* = 16) pairs that exhibited no response (*none*) or a response composed of a depolarization (*D*), a depolarization followed by a hyperpolarization (*DH*) or a hyperpolarization (*H*). Each graph illustrates the results for 1, 2, 5 and 10 spikes (*sp*) at 15Hz

The hyperpolarizing response reversed upon hyperpolarization of the T cell

The responses of T cells to P or N cells were studied as the T cells were set at different membrane potentials (Fig. 4A). These experiments revealed that the hyperpolarization reversed at around −40mV (Fig. 4B). Nicholls and Baylor (1968) showed that loading the T cells with chloride caused the reversal of the inhibitory responses. We confirmed this observation following a similar procedure (Fig. 4C). Taken together, these data strongly support the view that the inhibition was mediated by the activation of a chloride conductance.

The depolarizing component was revealed mainly as a suprathreshold response. The amplitude of the subthreshold expression of this depolarization was small, indicating that it was elicited at sites electrically distant from the soma but close to a spike initiation site. This condition precluded a reliable study of its reversal potential.
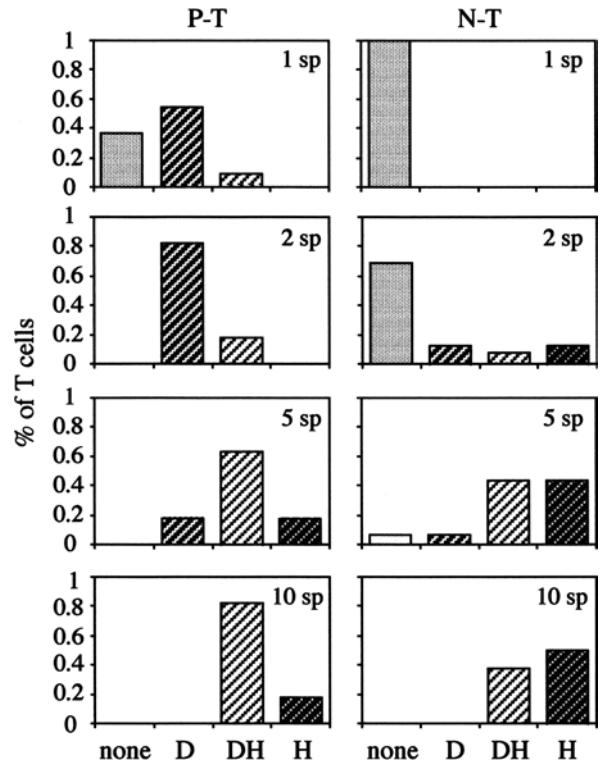
Synaptic connectivity between P, N and T cells

To examine whether the synaptic response was transmitted through mono- or polysynaptic pathways, experiments were performed in a solution containing $10 mmoll^{-1} Mg^{2+}$ and $10 mmoll^{-1} Ca^{2+}$, which impairs polysynaptic pathways in the leech by shifting the firing threshold of neurons to more positive values (Nicholls and Purves 1970). Figure 5 shows a representative example, in which the hyperpolarization induced by P cell stimulation was reversibly suppressed (*n* = 3) by the high divalent solution. The same was observed when N cells were stimulated (*n* = 3), suggesting that the responses of T cells were not due to direct interactions among the primary sensory neurons but to the activation of an interneuronal layer.

Activity in the P cells inhibit the responses of T cells to central activation

To investigate the influence exerted by P cells on the activation of T cells, we elicited the simultaneous activation of T and P cells. T cells were excited by central and peripheral stimulation of the neurons.
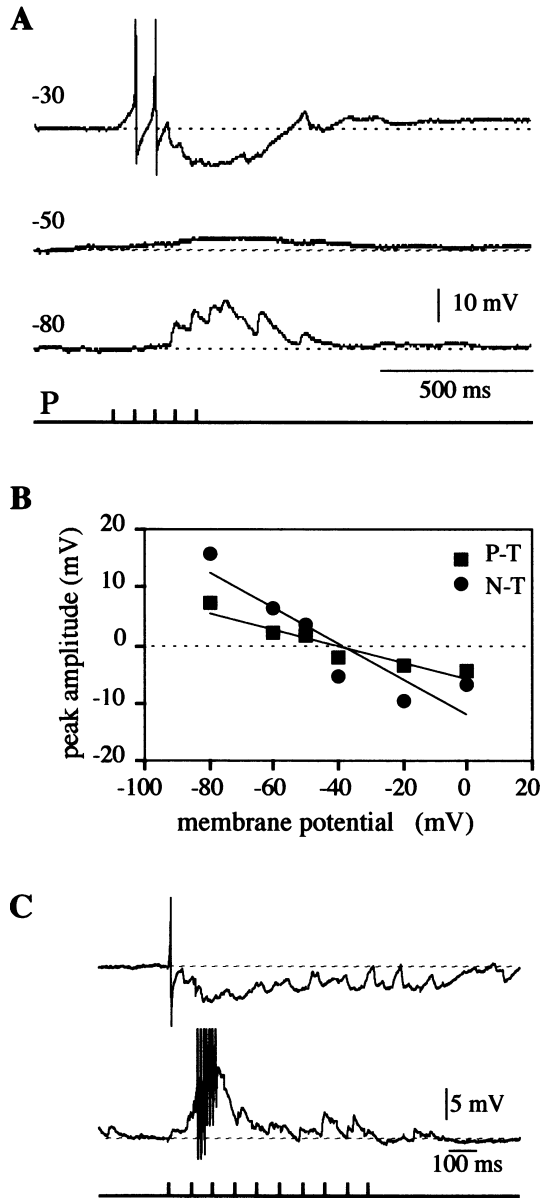
A

B

C

Fig. 4A–C Responses of T neurons held at different membrane potentials. **A** The traces show the intracellular recording of a T neuron as a P cell fired a train of five action potentials at 15Hz. The T cell was set at different membrane potentials, as indicated on the *left* of each trace (in mV). The *dotted lines* indicate the projection of the baseline before the stimulus. The *vertical lines* at the bottom of the traces indicate the timing of the action potentials in the P cell. **B** The graph shows the average peak amplitude of the suprathreshold responses of T neurons to stimulation of P ($n = 4$) or N ($n = 6$) cells, measured as a function of the membrane potential. The data was fitted to a linear function ($R = 0.9$ and 0.94 for P and N, respectively). **C** The recordings show the responses of a T cell impaled with an electrode filled with $3moll^{-1}$ KCl, 15s and 3min after impalement (*upper* and *lower* traces, respectively). The *vertical lines* at the bottom of the traces indicate the timing of the action potentials in the P cell

To activate the T cells centrally, prolonged (2s) steps of positive current (0.5–1nA) were injected in the soma of these neurons. Shortly before exciting the T cell, P cells were stimulated with a train of pulses that evoked
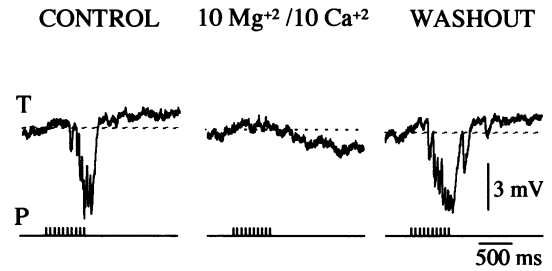
CONTROL   10 Mg^{+2} /10 Ca^{+2} WASHOUT

Fig. 5 Synaptic connectivity between P and T cells. Recordings of a T neuron during P cell stimulation studied, successively, in saline solution (*control*), after 3min superfusion with a $10mmoll^{-1}Mg^{2+}$/ $10mmoll^{-1}Ca^{2+}$ solution, and after 10min washout in saline solution. The *vertical lines* beneath the series of traces indicate the timing of the action potentials in the P cell

different number of spikes, at a frequency of 10Hz. Figure 6A shows representative responses to the application of this protocol. The prolonged current pulse injected in the soma of the T cells evoked a train of action potentials (Fig. 6A, panel a) that were quantified as the number of spikes during the step. Concomitant stimulation of a P cell inhibited the T cell firing to a degree proportional to the period of P cell firing (Figs. 6A, panels b–d; 6B).

Selective activation of T cells by water bubbling on the leech skin

Selective activation of mechanosensory T cells by mechanical stimulation of the skin requires the application of a light tactile stimulus (Nicholls and Baylor 1968). In search for a suitable stimulation to activate these mechanoreceptors in a reliable and standardized way we tested the ejection of air-pressure pulses on the skin, through a micropipette (see Materials and methods). The pressure pulses were directed at different skin fields–dorsal, ventral and lateral–along the central annulus, and produced maximal responses in specific T cells Td, Tv and Tl. Figure 7 shows a diagram of the body-wall preparation and four traces displaying the responses of one T cell that was recorded intracellularly, while the skin was stimulated at the sites indicated by the origin of the arrow lines. The recorded T cell corresponded to a Tv and had a maximal response to the stimulation of the ventral region of the skin.

The number of spikes, duration and latency of the trains varied among different preparations, but within each preparation the responses remained relatively constant upon successive identical stimuli (Fig. 8A). To quantify the stability of the responses we measured the number of T cell spikes produced by the stimulus and the latency to the first spike, along five successive stimuli applied at 1-min intervals. The results indicate that the number of spikes and latency remained relatively stable upon successive stimulation episodes (Fig. 8B).
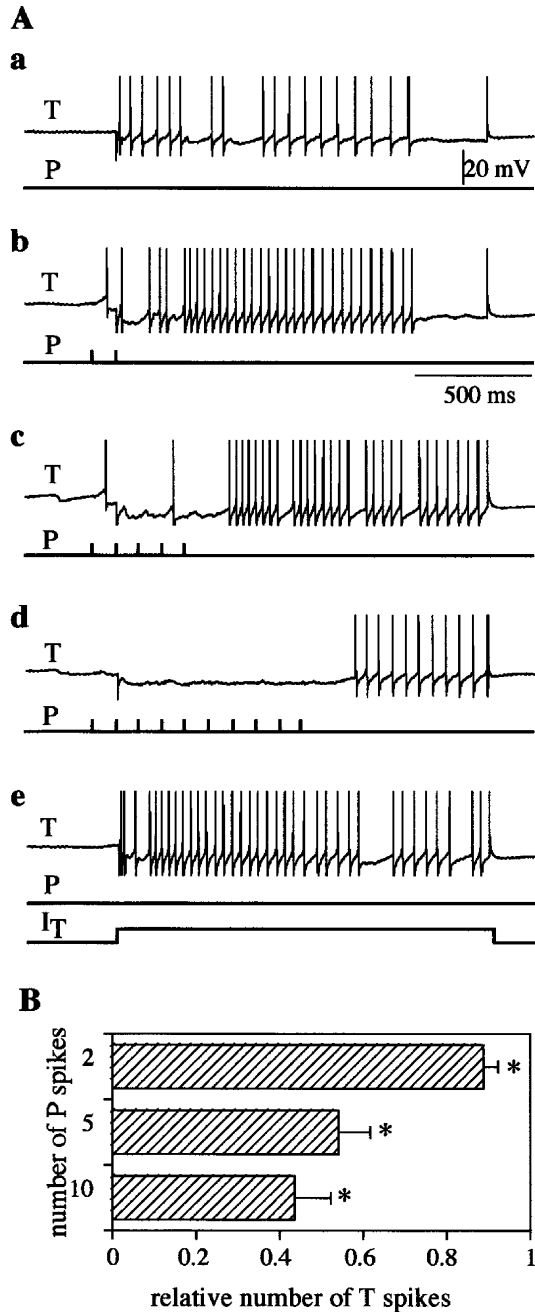
A

a

b

c

d

e

B

Fig. 6A,B Effect of the synaptic inhibition on T cell firing. **A** Recordings of a T cell during the injection of a current step of +0.5nA (I_T) as P remained at rest (*panels a* and *e*) or when it was stimulated with a train of two (*panel b*), five (*panel c*) and ten (*panel d*) spikes at 10Hz. The *vertical lines* underneath each recording indicate the timing of the P cell spikes and the square step at the bottom of the recordings indicates the duration of the current injection in T. **B** The *bars* indicate the average relative number of spikes in T cells ($n = 7$) during the depolarizing step, as P cells were induced to fire two, five and ten spikes. The values were relative to the mean of the initial (**A**, *panel a*) and the last (**A**, *panel e*) control. The ratio between the last and the initial control was of 1.06 ± 0.06. An *asterisk* indicates statistical significance ($P < 0.01$, paired *t*-test)

The mechanical stimulation evoked by the air pressure did not affect the other mechanosensory neurons, the P and the N cells (data not shown). Only rarely did P

cells fired an action potential at the end of the pulse, but this effect was not consistent in different trials.

To evaluate whether the responses of T cells to pulses of air pressure were due to a direct effect on the sensory terminals, rather than indirectly through the activation of the sensilla, we performed the experiments shown in Fig. 8 in a solution containing $20mmoll^{-1}$ Mg^{2+} and $1mmoll^{-1}$ Ca^{2+}, that blocks chemical synaptic interactions in the ganglion. The responses were effectively maintained in preparations that were bathed in the high Mg^{2+} concentrations for around 30min ($n = 3$), suggesting that the T cells were the primary sensory neurons affected by the stimulation pattern used.

Finally, this experimental scheme was employed to determine whether the hyperpolarization induced by P or N stimulation affected the response of the T cells to peripheral stimulation. Because stimulation of P or N cells evoked muscle contraction these experiments had to be performed under certain restrictions. The stimuli applied to P neurons had to be no longer than seven spikes, and at a maximal frequency of 10Hz. This stimuli were of similar characteristics as those applied in the isolated ganglia (Fig. 6). We did not stimulate N cells because they tended to produce very intense contractions.

Figure 9 displays representative recordings showing the electrophysiological activity of T cells when exerting separate and simultaneous stimulation of the skin and of P cells. In Fig. 9A the upper T trace shows the hyperpolarization induced by the stimulation of a P cell; the middle trace shows the excitation produced by skin stimulation; and the lower T trace shows the combination of both stimuli when the spiking of the P cell was evoked in the middle of the skin stimulus period. Figure 9B shows a similar experimental procedure, but the spiking of the P cell preceded the skin stimulus. In both cases the P cell stimulation was devoid of any effect on the response of the T cells to peripheral stimulation. To quantify the T-cell responses in the presence or absence of P stimulation we counted the number of spikes fired by the T cells within the period corresponding to the P stimuli. During co-activation of the P cell the number of spikes of the T cell was $94 \pm 6\%$ that recorded in the absence of P cell firing ($n = 6$).

Discussion

Synaptic interactions among the mechanosensory neurons

The present investigation supports and extends the conclusions obtained by Baylor and Nicholls (1969). The results show that in the leech the mechanosensory neurons with the highest sensory threshold (P and N cells) inhibited the mechanosensory neuron with the lowest sensory threshold (T cells), through the activation of a chloride conductance. The interactions between the

Fig. 7 Body-wall preparation. The scheme describes the body-wall preparation, where the *dark bands* represent the colored landmarks of the leech skin. A hole was made in the middle to uncover the ganglion. The *traces* underneath show four recordings of a T cell obtained by stimulating the skin (see Materials and methods) at the four sites indicated by the origin of the *arrow lines*
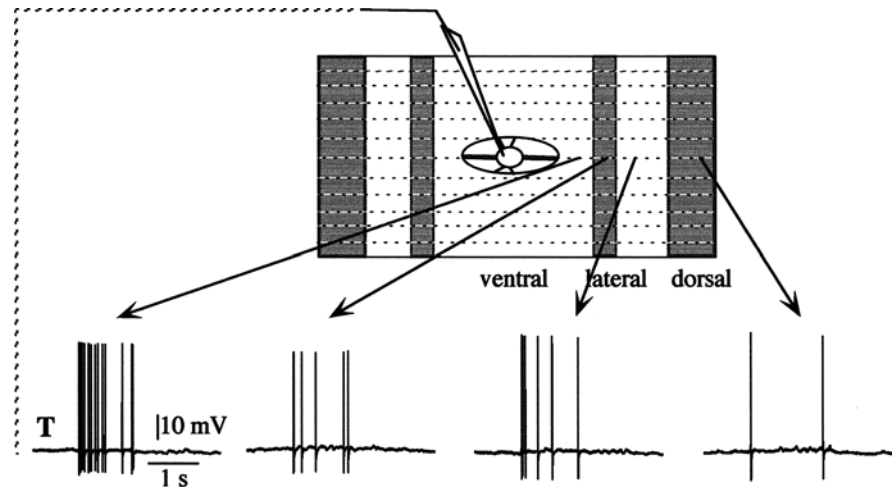
sensory neurons were mediated by interneuron(s), conforming a feedback network.

The excitation of P and N cells produced also a depolarizing phase, and the relative weight between the excitatory and inhibitory phases of the responses depended on the stimulus duration. One or two action potentials elicited in P neurons produced depolarizing responses in T cells, while longer stimuli evoked a clear hyperpolarization. N cells differed with respect to P cells, in that the contribution of the depolarization was significantly smaller. Thus, the sensory neuron with the highest threshold (N) produced predominantly inhibitory signals, while the sensory neuron with the intermediate threshold (P) could, upon a few spikes, excite the cell with the lowest threshold (T).

Central versus peripheral interactions

The double-stimulation experiments showed that the effectiveness of the inhibitory input from P depended on the site at which the firing was initiated. When the T cells were activated peripherally, by a stimulus exerted on the skin, the inhibition caused by P or N cells did not affect the activity of the T cells. However, when the T cell spikes were evoked centrally, the hyperpolarization induced by a similar P cell stimulation inhibited the T cell firing throughout the stimulus duration.

It has been reported that inhibitory synapses can be located at different sites of the neuronal architecture, producing differential effects on distal versus proximal inputs. In crayfish, the lateral giant neurons receive tonic inhibition distal, and recurrent inhibition proximal to the spike-initiation zone. Proximal inhibition was shown to act efficiently to prevent firing, while distal inhibition allowed a more subtle competition between excitation and inhibition (Vu et al. 1993; Edwards et al. 1999). In the electric fish, it has been suggested that distal and proximal inhibition to the electrosensory lateral line lobe served as low- and high-pass filters to the sensory input, respectively (Berman and Maler 1998).
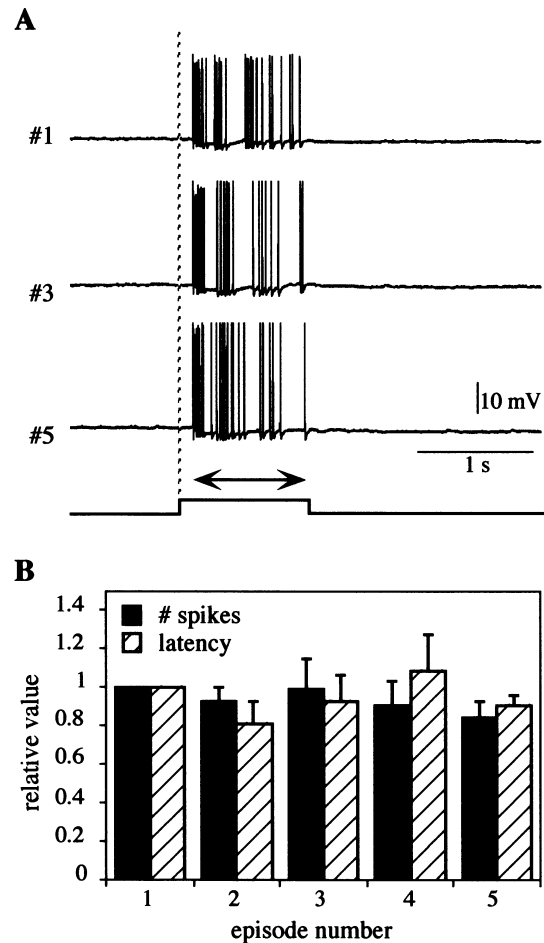

Fig. 8A,B The peripheral stimulation evoked a stable response in T cells. **A** The three traces show the responses of a T cell to the first (*#1*), third (*#3*) and fifth (*#5*) episode of five successive stimuli applied to the skin, at 1-min intervals. The duration of the pressure pulse is indicated by the *square step* at the bottom of the recordings. The *double arrowhead* line indicates the period in which the spikes where counted. The latency of the response was measured form the beginning of the pressure pulse (*broken vertical line*) to the first spike. **B** The *bars* indicate the mean relative number of spikes and the mean relative latency measured at each one of the five successive episodes. The values are expressed relative to those obtained at the first episode for each of four preparations analyzed
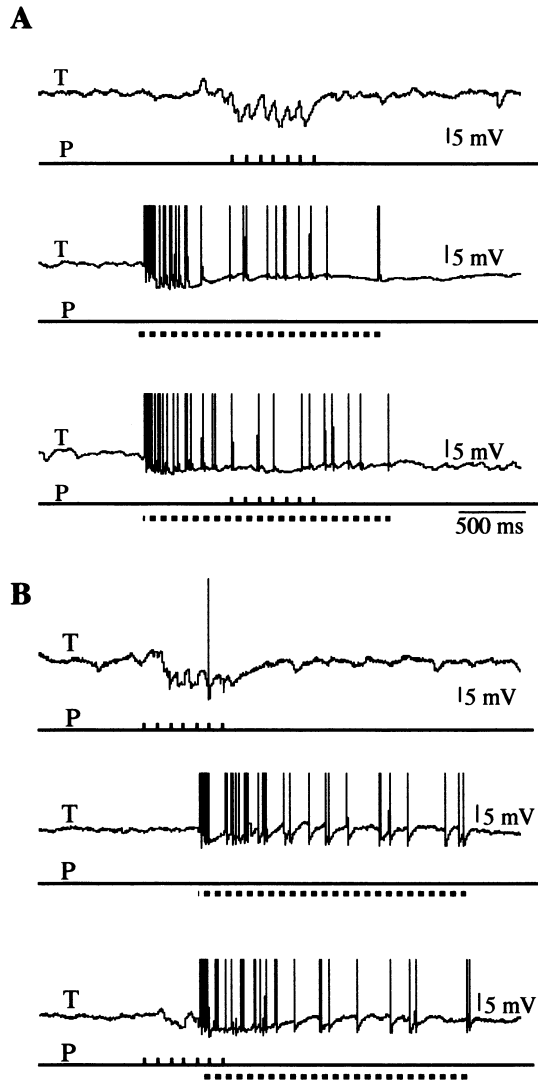
A

B

Fig. 9A,B Responses of a T cell to co-stimulation of the skin and the P cells. **A** The traces show the responses of a T cell to (from top to bottom): P cell stimulation (seven spikes at 10Hz), skin stimulation and P+skin stimulation. **B** As in **A**, but the P cell stimulation was applied with different timing. The *lower vertical bars* indicate the timing of the P cell spikes and the *thick dashed line* indicates the timing of the pressure pulse applied to the skin

Because the inhibition was observed in isolated ganglia we concluded that the inhibitory synaptic input impinged the T cells at the central branches of the cell arbor. Thus, taken together, our results indicate that intersensory interactions in the leech nervous system took place at a site electrically proximal to the soma and away from the spike initiation zone activated by peripheral stimulation. Thus, a strong competition between excitation of the different sensory neurons would probably be necessary to obtain sensory interference by co-activation of the neurons by their natural peripheral sources. However, selective activation of the P or N cells inhibited the T cells at the central nervous system and could prevent activation of the latter by inputs acting centrally. The scheme shown in Fig. 10 summarizes these considerations.
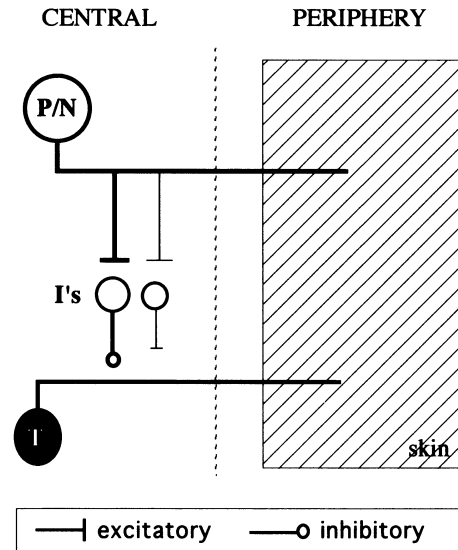

Fig. 10 Interactions among mechanosensory neurons. The diagram summarizes the connectivity of P or N with T cells. Each neuron extends projections to the periphery that innervate the skin. Stimulation of P or N activated excitatory and inhibitory interneurons (*I's*) at central sites, but the inhibitory input was much more preponderant. The *line thickness* indicates the weight of the connections

Physiological implications

Notwithstanding these limitations, it is possible to conclude that stimuli that evoked moderate responses in the P cells would not affect the concomitant responsiveness of T cells to those stimuli but, instead, they would influence the neuronal activity initiated centrally. T cells in the same hemiganglion and in adjacent ipsilateral hemiganglia are linked through a network that enables reverberation of their electrical activity (Mistick 1978). The inhibitory input from P and N cells could serve as a mechanism to set bounds to the activity initiated by stimuli that would be strong enough to excite T and P/N cells.

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