Functional Significance of Passive and Active Dendritic Properties in the Synaptic Integration by an Identified Nonspiking Interneuron of Crayfish

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Abbreviated title: Synaptic integration in nonspiking interneuron

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Abstract

Nonspiking interneurons control their synaptic output directly by membrane potential changes caused by synaptic activities. Although these interneurons do not generate spikes, their dendritic membrane is endowed with a variety of voltage-dependent conductances whose functional significance in synaptic integration remains unknown. We quantitatively investigated how the passive and active dendritic properties affect the synaptic integration in an identified nonspiking interneuron of crayfish by computer simulation using its multicompartment model based on electrophysiological measurements and three-dimensional morphometry. At the resting potential level, the attenuation factor (Vs/Vt) of a unitary synaptic potential in the course of its spread from a dendritic terminal (Vs) to other terminals (Vt) ranged 4.42 - 6.30 with no substantial difference between hyperpolarizing and depolarizing potentials. The compound synaptic responses to strong mechanosensory stimulation could be reproduced in calculation only as the result of spatial summation of attenuated potentials, not as any single large potential. The characteristic response could be reproduced by assuming that the active conductances were distributed only in the dendritic region where the synaptic summation was carried out. The active conductances in other parts of the cell affected neither the shape of the compound synaptic response nor the dendritic spread of synaptic potentials. These findings suggest that the active membrane conductances do not affect the spatial distribution of synaptic potentials over dendrites but function in sculpting the summed synaptic potential to enhance temporal resolution in the synaptic output of the nonspiking interneuron.
INTRODUCTION

Nonspiking interneurons exert graded and continuous output on postsynaptic cells without generating spikes. They are involved in the neuronal circuit for sensory information processing (DiCaprio, 2003; Marder and Bucher, 2001) and motor control (Hama and Takahata, 2005; Murayama and Takahata, 1998) systems of arthropods. For example, the LDS interneuron identified in the terminal abdominal ganglion of crayfish (Reichert et al., 1982) is involved in the mechanosensory information processing of the tailfan, receiving depolarizing monosynaptic inputs from water current-sensitive cuticular hairs on the soma side to mediate the lateral inhibition of ascending interneurons on the opposite side so that their directional sensitivity is enhanced (Krenz and Reichert, 1985; Reichert et al., 1983). Nonspiking, graded synaptic transmission is also known in the annelid nervous system (Angstadt and Calabrese, 1991). The output synapses of nonspiking interneurons can continuously release neurotransmitters depending on the membrane potential that is controlled in a graded way by synaptic activities (Burrows, 1979; Burrows and Siegler, 1978) and are located dispersively on the fine distal branches all over the entire dendrite, intermingled with input synapses (Kondoh and Hisada, 1986a, b; Watson and Burrows, 1988). Hence the possibility proposed in the central neurons of vertebrates that independent input-output processing units, or dendritic "subunits", operate in parallel within a single neuron (Shepherd, 2004) can be also proposed in nonspiking interneurons (Koch and Segev, 1998; Pearson, 1976; Wilson and Phillips, 1983). However, almost no experimental or theoretical support is available for this possibility except the steady-state analyses conducted by Rall (1981).

The physiological process of synaptic integration depends on the passive electrotonic structure and active membrane properties of neuronal dendrites (Koch and Segev, 2000;
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London and Häusser, 2005; Williams and Stuart, 2003). A typical central neuron receives many synaptic inputs and develops a membrane potential change by summing them up to generate an output signal in the form of a spike train that is usually transmitted to postsynaptic cells by way of a single process. Hence the most important there is how the membrane potential of the spike initiating region changes as a consequence of the summatory interaction of synaptic inputs. Accordingly, previous analyses have mostly focused on how the synaptic events affect the membrane potential of the spike initiating region (e.g., Gabbiani et al., 2004 for an insect visual interneuron; Polsky et al., 2004 for vertebrate pyramidal cells). In nonspiking interneurons, however, the spatio-temporal distribution of synaptic potentials over the whole dendrite is important for understanding their synaptic integration capabilities since the output sites of these interneurons are potentially distributed all over the fine dendritic branches (Kondoh and Hisada, 1986a, b; Watson and Burrows, 1988).

Nonspiking interneurons are also known to have a variety of voltage-dependent membrane conductances. In locust, they have depolarization-dependent, transient and delayed potassium conductances on their neuropilar membrane (Laurent, 1990, 1991) as well as a depolarization-sensitive transient calcium conductance on the isolated soma membrane (Laurent et al., 1993). We showed in crayfish that the LDS interneuron have three distinctive active conductances: a sustained component and two kinds of transient components (Takashima and Takahata, 2000). By experimental analyses using intracellular techniques (Takahashi and Takahata, 1995) and computer simulation based on a single compartment model (Takahata et al., 2000), these conductances were shown to have significant effects on the time course of synaptic potentials. The LDS interneuron, however, has extensive dendrites that make fine arborizations on both sides of the ganglion,
receiving mechanosensory input on the soma side and exerting inhibitory output on the opposite side. Hence, quantitative knowledge on how the active membrane conductances affect the spatial distribution of synaptic potentials over dendrites is crucial for understanding the integrative characteristics of nonspiking interneurons.

In the present study, we addressed a question of how the spatio-temporal distribution of synaptic potentials over dendrites of the LDS interneuron is affected by passive and active membrane properties as well as its dendritic morphology. We constructed a multicomartment model of the interneuron based on current- and voltage clamp experiments (Takashima and Takahata, 2000) and three-dimensional morphometry using a confocal laser scanning microscope (Hikosaka and Takahata, 1998). The model was verified by comparing the calculated results with experimentally obtained data. We show that summation of synaptic input and distribution of the summed activity to output synapses are primarily dependent on the electrotonic structure of dendrites whereas the summed potential is critically deformed by active membrane conductances in the LDS interneuron.
METHODS

Electrophysiology

Adult crayfish, Procambarus clarkii Girard, of both sexes ranging 7 - 10 cm in body length were used. The abdominal nerve cord including the terminal abdominal ganglion was isolated from the rest of the body and pinned to a silicone elastomer-lined chamber with its dorsal side up. The chamber was filled with crayfish saline. All electrophysiological experiments were carried out with a single borosilicate glass microelectrode filled with 3 M KCl or 3 % Lucifer yellow in 1 M LiCl. Intracellular recording was made from the thick transverse segment of the interneuron on or near the midline to keep the damage due to electrode penetration as small as possible. Details of electrophysiological experiments are described in a previous paper (Takashima and Takahata, 2000). Intracellular staining was performed by iontophoretic injection of Lucifer yellow (10 nA hyperpolarizing current, 500 msec duration, 1 Hz). For further staining of the sensory nerve fibers, the cut end of the second or third root of the terminal abdominal ganglion was immersed in a small well filled with 10 mM ethydium bromide/1 M KCl solution and the whole preparation was kept in a refrigerator for 4 hours. The preparation was fixed in 10% formalin for 5 minutes, dehydrated in 100% ethanol for 5 minutes, cleared in methylsalicylate, and examined by a fluorescence microscope (Olympus BHS-RFC) or a confocal laser scanning microscope system (Molecular Dynamics, Sarastro 2000). Details of morphological measurement in the three-dimensional space are described in previous papers (Hikosaka and Takahata, 1998, 2001; Hikosaka et al., 1996).

Multicompartmental Modeling

The whole neuron was represented by an assembly of 493 cylindrical compartments
each having specific diameter and length values (Edwards and Mulloney, 1987; Perkel et al., 1981; Segev et al., 1998) except the cell body that was represented by an ellipsoid. Each compartment was connected with its neighbors with axial resistances. The electrotonic length of a single compartment was chosen to be less than 0.1$\lambda$, where $\lambda$ is the length constant, so that each compartment could be regarded as isopotential. $\lambda$ was obtained using the following relationship,

$$\lambda = \sqrt{\frac{DR_m}{4Ra}}$$

where D is the dendritic diameter obtained by morphological measurement, $R_a$ the axoplasm resistivity assumed to be 0.06 k$\Omega$cm (Rall, 1981). $R_m$, the membrane resistance per unit area, was obtained by electrophysiological measurement of the membrane time constant $\tau$ using the following relationship,

$$\tau_m = R_mC_m$$

where $C_m$ is the membrane capacitance per unit area assumed to be 1 $\mu$F/cm$^2$ (Koch, 1999; Rall, 1969). Experimental procedures for obtaining the membrane time constant from the voltage response to step current injection are described elsewhere in detail (Takahashi et al., 1995). In the cell that was used to construct the multicompartment model for the present study, the experimentally obtained value for $\tau_m$ was 25.8 msec, yielding the membrane resistance of 25.8 k$\Omega$cm$^2$, as measured in the thick transverse segment over the midline. $R_m$ was assumed to be uniform over the whole neuron. Also incorporated into the present model were three kinds of depolarization-dependent outward conductances, i.e., a sustained ($g_s$) and two types of transient ($g_{t1}, g_{t2}$) conductances, as well as a leak conductance ($g_{leak}$). Details on numerical reconstruction of these conductances based on Hodgkin and Huxley (1952) were described in a previous paper (Takashima and Takahata,
Experimental analyses have suggested that all of three kinds of voltage-dependent currents are carried by potassium ions. Thus the whole ionic current flowing through the membrane including the leak current is expressed as

\[ I_m = g_s(V-E_K)+g_{t1}(V-E_K)+g_{t2}(V-E_K)+g_{\text{leak}}(V-E_{\text{leak}}) \]  

(eq. 1)

in which \( E_K \) and \( E_{\text{leak}} \) are the equilibrium potentials for outward and leak current respectively. \( E_K \) was -70.0 mV according to the experimental data. \( E_{\text{leak}} \) was assumed to be the same as the resting potential. The voltage- and time-dependent parameters for the three potassium conductances (\( g_s, g_{t1} \) and \( g_{t2} \)) were based on our previous experimental data (Takashima and Takahata, 2000; Table 1). The membrane potential of the \( i \)th compartment (\( V_i \)) as a function of time was therefore obtained by numerically solving a set of first-order ordinary differential equations

\[ \frac{dV_i}{dt} = \frac{I_{\text{inj}}-I_m-\sum_{j=1}^{N} \frac{V_j-V_i}{r_{ij}}}{C_m} \]  

(eq. 2)

in which \( C_m \) represents the total membrane capacitance calculated from the membrane area, \( I_{\text{inj}} \) the current injected intracellularly, \( r \) the axial resistances connecting the compartment with its \( N \) neighbors.

The voltage-dependent potassium conductances were numerically reconstructed according to Hodgkin-Huxley (1952) by fitting experimental results to the general form of equation:

\[ g = \overline{g} m^p h^q \]

where \( g \) is the membrane conductance and \( \overline{g} \) is the maximal membrane conductance for the potassium ion. The values for \( g \) and \( \overline{g} \) were obtained experimentally (Takashima and Takahata, 2000). \( m \) and \( h \) are voltage- and time-dependent gating variables for activation
and inactivation respectively, ranging between 0 and 1. The gating variable dynamics were modeled as the solution to the equation

\[ \frac{dm}{dt} = \alpha_m(1-m) - \beta_m m \]

in which \( \alpha_m \) and \( \beta_m \) are voltage-dependent rate constants for transition of the gate to permissive and non-permissive state respectively (Nelson and Rinzel, 1997) based on the assumption that the transition obeys first-order kinetics. \( \alpha_m \) and \( \beta_m \) in the present study were described by either exponential or sigmoid function of voltage in the following general forms:

\[
\alpha_m(V) = \frac{A}{B + \exp\left(\frac{V + C}{D}\right)} \\
\beta_m(V) = \frac{E}{F + \exp\left(\frac{V + G}{H}\right)}.
\]

Values for parameters A - H, determined on the basis of our experimental data (Takashima and Takahata, 2000), are listed in Table 1. These values were fixed throughout this study. The model was tuned by changing the membrane area of the cell body and dendrites as described below.

**Verification of the Constructed Model**

The constructed model was verified by comparing the recorded response to a step hyperpolarizing current (1 nA) injected into the thick transverse segment with the calculated response of the model to the same current injection into a compartment that corresponded to the transverse segment. In constructing the model, the results of three-dimensional morphometry on the compartmental diameter were multiplied with 1.2 to compensate for the shrinkage due to fixation and clearing procedures that followed fixation (Hikosaka et al., 1996). It was found, however, even after this shrinkage compensation, the calculated response of the model was significantly larger (> 136.8%)
than the recorded one, suggesting that the total membrane area in the morphological measurement was underestimated. This underestimation appears to be partly due to the current procedure that each dendritic branch was approximated by one or more cylindrical compartments. It was also likely that those processes with diameters less than 1 \( \mu m \) might have eluded the present measurement that was limited by the spatial resolution of the confocal laser scanning microscope system (Hikosaka and Takahata, 1998; Hikosaka et al., 1996). In addition, electronmicroscopic studies have revealed that the membrane of dendritic branches is not smooth but generally shows irregular profiles (Kondoh and Hisada, 1986a, b). In particular, it is well known that the soma membrane of invertebrate central neurons shows extensive folding structure (Kondoh and Hisada, 1986a, b; Watson and Burrows, 1982, 1983). The soma surface area may be at least four times larger than the estimated value in a cylinder or sphere having a comparable volume with the soma (Watson and Burrows 1983). We therefore limited the soma surface compensation factor to the order of 4 - 6, and then changed the dendritic surface compensation factor to obtain the best fit. Calculation of the residual sum helped this adjustment, but the final decision was made on the basis of visual best fit. In the present model, the soma and dendritic membrane area was assumed to be 5 times and 1.27 times larger than the measurement respectively without a change in the axial resistance so that the recorded and calculated responses were made comparable.

**Simulation of Synaptic Activities**

Simultaneous intracellular staining of the LDS interneuron and its presynaptic mechanosensory afferents in the same preparation (Fig. 1A) has suggested that the afferent fibers make synaptic contacts with the interneuron at its distal dendrites. This observation
is consistent with the result of electronmicroscopic study that input synapses of the LDS interneuron are mostly distributed on fine branches rather than on thick ones (Kondoh and Hisada, 1986a). In this study, one or several branch terminals were assumed to bear synaptic sites receiving the afferent input (Fig. 1B). Synaptic responses of the model cell were calculated by adding a term representing the synaptic current in the form of $g_{syn}(V_i - E_{syn})$ to the equation for $I_m$ (eq. 1). $g_{syn}$ is the synaptic conductance and $E_{syn}$ the reversal potential that is assumed to be 0 mV for the excitatory input and -70 mV for the inhibitory input. $g_{syn}$ is assumed to follow the alpha function (Jack et al., 1983) with the peak time of 1.0 msec. The maximal synaptic conductance and the number of compartments bearing the input synapse were adjusted so that the potential change due to a single synaptic input and the compound response to multiple input were of comparable magnitude with the actual synaptic potentials recorded in physiological experiments.

Simulations were performed with GENESIS (Wilson et al. 1989) on a Pentium IV-class PC by solving the simultaneous equations (eq. 2) numerically. For integration, we used the Hines algorithm (Hines 1984) available in GENESIS version 2.0 (Bhalla al. 1992). Simulations were run with a time step of 0.02 msec. Initial control simulations using 0.01 msec showed that the 0.02 msec step produced numerically accurate results. For the model presented here, 200 msec of the LDS cell activity could be simulated in 5 min. Several hundred simulations were run, mostly to explore appropriate parameters.
RESULTS

We constructed a family of models, each with 493 compartments, that differed in the membrane area of the cell body and dendrites as well as in the distribution of active conductances among different compartments. Other parameters of the model were all based on experimental data. We tuned each model's membrane area and conductances by fitting to physiological data. We will first summarize them briefly in the following section.

The LDS interneuron extends dendrites on both sides of the terminal abdominal ganglion. Branches on each side are connected by a thick transverse segment over the midline in the dorsal half of the ganglion (Fig. 1A). The cell body is located ventro-medially in the middle of the ganglion and connected to the branches on the same side. When recorded in the transverse segment, the interneuron spontaneously shows depolarizing and hyperpolarizing synaptic potentials at the resting potential level (Fig. 2A). They are separated from each other in most cases, with no apparent summation nor interaction, so that they can be discriminated individually. It can be seen in the record that not only the peak amplitude but also the time course is different between depolarizing and hyperpolarizing synaptic potentials: depolarizing potentials are larger and faster than hyperpolarizing ones. The rise rate was $0.43 \pm 0.04 \text{ mV/msec}$ (mean $\pm$ standard error) and the half decay time was $15.70 \pm 0.93 \text{ msec}$ in the depolarizing ($N = 27$) whereas they were $0.15 \pm 0.01 \text{ mV/msec}$ and $23.60 \pm 1.90 \text{ msec}$ in the hyperpolarizing potentials ($N = 24$; Fig. 2B). These differences were statistically significant ($P < 0.001$; Student's two-sided t-test).

Moreover, the depolarizing response of the LDS interneuron evoked by electrical stimulation of the sensory afferent bundle showed rapid repolarization in its initial phase followed by a slowly waning component when the response became larger than about 20 mV in amplitude (Fig. 2C). In response to step current injection, the dendritic membrane of
the LDS interneuron showed outward rectification when it was depolarized by more than 10 mV from the resting potential (Fig. 2D). These findings indicate that, due to the depolarization-dependent membrane rectification, the synaptic input to the interneuron is processed quite differently depending on its polarity as well as the membrane potential level at which it is evoked. The rapid repolarization as observed in the synaptic response (Fig. 2C) was less remarkable in the current injection experiment (Fig. 2D), but was still discernible as indicated by an arrowhead. These transient peaks were consistently observed in the calculated responses (Fig. 2E arrowhead). They were variable in the experiment, however, from preparation to preparation and even within a preparation depending on the time, probably due to membrane damages caused by microelectrode penetration and to deterioration of the preparation. They were clearly seen in another experiment (Fig. 2F).

Electrotonic Spread of Hyperpolarizing Potentials over Dendrites

Previous studies have revealed that the dendritic membrane of the LDS interneuron behaves passively at and below the resting potential level (Takahashi et al., 1995; Takahata et al., 1995; Takashima and Takahata, 2000). The validity of the present model was verified by comparing experimentally recorded and computationally calculated passive responses in the same dendritic region. The cell was impaled with a microelectrode in the region b shown in Fig. 1B. After appropriate modification of structural data (see Methods section), the recorded response of the cell to a hyperpolarizing current step (1 nA) was in good agreement with the calculated response of the cell (blue dotted line in Fig. 3A) to the same amount of current injected in a compartment that corresponded to the region b in Fig. 1B. We could obtain neuron models that showed better agreement with the recorded response (red dotted line in Fig. 3A) by assuming larger values for the cell body surface area, but we
did not adopt them in this study since those values were unrealistic (see Methods section).
It should be noted here, however, that the calculated synaptic response was almost the
same regardless of the assumed soma surface area if it was more than five times larger.

Using this passive model of the LDS interneuron, we analyzed the spatio-temporal
distribution of hyperpolarizing synaptic potentials over the dendrite. Electrical stimulation
of a ganglionic root on the side opposite to the soma evokes a small depolarizing response
followed by a slow hyperpolarizing response that interacts with the depolarizing input from
the soma side to reduce it effectively (Takahata et al., 1985), suggesting that the directional
sensitivity of the tailfan mechanosensory system is more enhanced by this inhibitory
pathway. The hyperpolarizing synaptic activity thus has a crucial significance in the
functioning of the LDS interneuron. We studied how a discrete hyperpolarizing potential
(Fig. 2A) spread over dendrites after generated by a single, but not collective, activity of
a central interneuron involved in the inhibitory pathway from the contralateral sensory
afferents to the LDS interneuron.

Following Kondoh and Hisada (1986a), we assumed in this calculation that an input
synapse was located at one dendritic terminal (shown with a single asterisk in Fig. 1B).
The conductance change was adjusted so that the amplitude of the calculated synaptic
potential in the transverse segment was comparable with that recorded in the
electrophysiological experiment. The shape of a single synaptic potential was calculated in
5 representative regions of the LDS interneuron including its cell body (a - d in Fig. 1B).
Not only the peak amplitude was greatly reduced but also their time course was
significantly expanded when the synaptic potential spread to the region a just proximal to
the synaptic site (Fig. 3B). It was further deformed when it spread from a to the transverse
segment b. No significant change was observed, however, in the peak amplitude and the
time course of the synaptic potential when it further invaded contralateral branches to their terminal (c, d). The membrane potential change of the soma was the smallest in amplitude and the slowest in time course. When the synaptic input came successively, a saw-toothed membrane potential change was produced in the synaptic region (Fig. 3C). This discontinuous potential was much more smoothed in other terminal regions since individual synaptic potential made temporal summation with each other to develop a sustained hyperpolarization. The soma showed almost a single slow response to the successive input.

The interneuron had 30 and 17 dendritic terminals on the side ipsilateral and contralateral to the soma respectively. In this study, we defined the attenuation factor for the voltage spread from the synaptic site (Vs) to another terminal (Vt) as Vs/Vt. It was 5.74 ± 0.09 (N = 29; mean ± SE) and 6.10 ± 0.01 (N = 17) for ipsilateral and contralateral branches respectively. The expansion factor for the half decay time (T) during the voltage spread from the synaptic site (Ts) to another terminal (Tt) was defined as Ts/Tt in this study. It was 0.21 ± 0.003 and 0.20 ± 0.001 (mean ± SE) for ipsilateral and contralateral branches respectively. The differences in the attenuation factor were statistically not significant (Student's two-sided t-test; P > 0.05). We conclude that the passive spread of a hyperpolarizing synaptic potential to ipsilateral terminals is not significantly different from that to contralateral ones. When a hyperpolarizing synaptic potential spread from the synaptic region (Vs) at the dendritic terminal on the soma side to the transverse segment (Vts) crossing the midline, the attenuation factor (Vs/Vts) varied with the terminal depending on the distance from the segment (Fig. 3D). It ranged from 2.26 to 12.93 with the mean value of 6.95 ± 0.54 (N = 29). The expansion factor ranged from 0.17 to 0.28 with the mean value of 0.21 ± 0.003 (N = 29).
Dendritic Spread of Depolarizing Potentials on the Active Membrane

We found that the excitatory synaptic potential behaved in the way similar to the inhibitory one when examined in the same five regions chosen for passive analyses (Fig. 1B) after adjusting the synaptic conductance change to make the amplitude of the calculated synaptic potential comparable with that of the recorded one. The peak amplitude was greatly reduced and their time course was significantly expanded in the region a (black line Fig. 4A). The amount and time course of potassium conductances activated by the potential also changed accordingly (blue line). The synaptic potential was further deformed when it spread from a to the transverse segment b, but almost no significant change was observed in the peak amplitude and the time course of the synaptic potential when it further invaded contralateral branches to their terminal (c, d). When a depolarizing synaptic potential spread from the synaptic region at one dendritic terminal (Vs) to other terminals (Vt), the peak attenuation factor (Vs/Vt) was 5.85 ± 0.10 (N = 29) and 6.24 ± 0.008 (N = 17) on the side ipsilateral and contralateral to the cell body respectively (P < 0.01) whereas the expansion factor for the half-decay time was 0.19 ± 0.003 (N = 29) and 0.19 ± 0.001 (N = 17; P > 0.05). When a depolarizing synaptic potential spread from the synaptic region at the dendritic terminal on the soma side (Vs) to the transverse segment crossing the midline (Vts), the peak attenuation factor (Vs/Vts) was 7.22 ± 0.58 (N = 29) ranging from 2.29 to 13.84 whereas the expansion factor for the half-decay time was 0.22 ± 0.01 (N = 29) ranging from 0.19 to 0.32 (Fig. 4B).

The voltage response was dependent on the synaptic conductance change: the response became greater as the conductance change increased (Fig. 4C). It is interesting to note here that the single large potential was affected by active membrane conductances in a
different way depending on whether the terminal compartment was connected to or isolated from the rest of the cell model. Thus, the repolarization was rapid on the whole when the compartment on which the excitatory synapse was assumed was connected with the cell (Fig. 4C). When the compartment was isolated from the rest of the interneuron, by contrast, it was rapid only at the early phase of the synaptic potential that was caused by less amount of conductance change for producing a depolarization comparable with that observed before (Fig. 4D). It is also noted here that the rise rate of the synaptic potential in the isolated condition was slower than in the connected condition. Thus, even though the dendritic membrane of the LDS interneuron is provided with depolarization-dependent membrane conductances, they affect the shape of synaptic potentials not unconditionally but dependently on the dendritic conformation.

When recorded in the transverse segment on the midline, the LDS interneuron shows characteristic responses to electrical stimulation of the sensory nerve bundle of the third ganglionic root (Reichert et al., 1983). As the stimulus intensity increases, the depolarizing synaptic response becomes larger in its peak amplitude, and finally the falling phase becomes steeper to show a narrow peak followed by a shoulder-like slow decay (Fig. 5A). In order to reproduce this characteristic response pattern, we had to assume that many peripheral compartments received excitatory synaptic input simultaneously. Assuming the synaptic input on only one terminal compartment, the membrane potential change due to synaptic activity was very small when recorded in the transverse segment (less than 1 mV) even though the conductance change was adjusted so as to produce a large synaptic potential at the terminal (Fig. 4A, B). In the simulation shown in Fig. 5B, the excitatory synapse was assumed on 94 compartments (shown with black in Fig. 1B) including not only terminal compartments but those located adjacent to or near the terminal. An increase
in stimulus intensity was modeled in this simulation by an increase in the number of activated compartment to rise the amount of synaptic currents. As the number of activated synaptic currents increased, the response as the sum of each small synaptic activity became greater, and finally it showed a steep and narrow peak (Fig. 5B) that was characteristic to natural responses (Figs. 2C, 5A). Since the afferent fibers appear to make contact with the LDS interneuron at many sites (Fig. 1A), we consider the present assumption reasonable, but the present assumption on the synaptic sites is not necessarily unique: the characteristic response pattern can be reproduced by assuming synapses on other sets of compartments. The crux is that the compound synaptic potential evoked by electrical stimulation of the sensory nerve bundle with increasing stimulus intensities can only be reproduced in simulation by assuming extensively dispersed synapses on peripheral compartments. With the depolarization-dependent conductances disabled, the calculated responses were larger in amplitude and slower in time course than those with active conductances enabled (Fig. 5C).

The characteristic response pattern of the interneuron showing a narrow peak in the large compound potential was not always the same; the time width of the peak varied from preparation to preparation as well as the voltage level at which the shoulder in the falling phase appeared (Fig. 2C, Fig. 5A, D1). In some preparations, the narrow peak was completely absent even when the stimulus intensity was raised to the maximum (Fig. 5D2). It is interesting to note here that the recorded responses shown in Fig. 5D2 look similar to those calculated with conductances disabled (Fig. 5C). These findings suggest that the variability among preparations in the synaptic response of the LDS interneuron to sensory bundle stimulation (Figs. 2C, 5A, D1-2) is caused by partial failure of depolarization-dependent membrane conductances due to experimental procedures. An
alternative possibility is that the variability is intrinsic and not caused artificially. In any case, the effect of depolarization-dependent conductances was more significant when the synaptic input was provided successively. When the stimulus intensity was low, i.e., when the number of activated current was small, individual synaptic activity was small so that the conductances were not fully activated. With the active conductances enabled, however, each synaptic event was more clearly evident (Fig. 5E). When the stimulus intensity was raised so that the synaptic response became larger to fully activate the depolarization-dependent conductances, the discreteness of each synaptic activity was more prominent than ever (Fig. 5F). Thus the depolarization-dependent membrane conductances serve for locking up each synaptic activity in a discrete form during repetitive activation to increase the temporal resolution of the tail mechanosensory system (see Discussion). It should be noted here that the large synaptic response including the transient component is caused artificially by simultaneous activation of all sensory afferents. This situation hardly occurs under natural conditions. Our calculation with a 100 Hz train of stimuli was intended to show the potential effect of potassium conductances on the temporal resolution in synaptic integration of the LDS interneuron.

Effects of Non-uniform Distribution of Active Conductances over Dendrites

Our finding that the characteristic time course of the compound depolarizing potential could be reproduced by spatial summation of many small depolarization (Fig. 5B), each of which was under the threshold for the conductances to be fully activated, suggested that the characteristic time course of the compound synaptic potential could be realized by localized distribution of active conductances at the site of summation, i.e., the transverse segment on the midline. We tested this possibility by constructing a model in which the
depolarization-dependent membrane conductances were implemented only to the transverse segment; the membrane behaved passively in other parts of the cell including the synaptic terminal. The total conductance change was the same as the experimentally obtained value in both the uniform and non-uniform models.

Calculation of a large compound synaptic potential in response to simultaneous activation of excitatory synapses revealed that its time course was similar regardless of whether the active membrane conductances were distributed uniformly (Fig. 6A1) or locally (Fig. 6A2). The transition from slow to fast response time course as the number of synaptic input increased was also comparable between the uniform and non-uniform model: the summed synaptic activity was slow and smooth in its time course reflecting the passive membrane properties as long as it remained under the threshold for activation of depolarization-dependent conductances, but became steeply deformed by them when it exceeded the threshold.

The spread of the compound synaptic potential from the transverse segment to output terminals on the dendrites contralateral to the soma were not affected by the depolarization-dependent conductances. In the uniform model (Fig. 6B1), the attenuation factor when the synaptic potential arrived at the output terminal $d$ shown in Fig. 1B was $1.12 \pm 0.003$ (N = 17) ranging 1.08 - 1.14 while the expansion factor was $0.89 \pm 0.01$ (N = 17) ranging 0.82 - 0.90. Those values were $1.15 \pm 0.004$ (N = 17) ranging 1.10 - 1.17 and $0.84 \pm 0.01$ (N = 17) ranging 0.82 - 0.90 respectively in the non-uniform model (Fig. 6B2) in which only the membrane of the transverse segment behaved actively. Both values showed statistical differences between the uniform and non-uniform models (P < 0.01). Considering the fluctuation of membrane potential due to synaptic noises (Fig. 2A), however, the differences are unlikely to have any physiological significance. Thus, for both
small (Fig. 4) and large (Fig. 6) depolarizing synaptic potentials, their transmission is almost solely dependent on the electrotonic structure of dendrites, irrespective of whether active membrane conductances are present or not in the membrane on the way from the integrating transverse segment to the synaptic terminals. Calculation of a single synaptic potential at the synaptic site (\* in Fig. 1B) and the midline region (a) in the uniform and non-uniform model revealed that both attenuation of the peak amplitude and expansion of the time course were comparable regardless of the distribution pattern of active conductances (Fig. 6A1 inset).

Simulation of Discrete Synaptic Potentials

Since the peak amplitude of the depolarizing potential was small (Fig. 2A), it was expected that the depolarization-dependent membrane conductances would have little influence on the shape of these synaptic potentials. However, the calculation assuming an input synapse on one dendritic terminal chosen arbitrarily revealed that the result of calculation in the transverse segment fit to the experimentally obtained record better with the active conductances incorporated into the model than without them (Fig. 7A). The hyperpolarizing synaptic potential was also found to be affected by active membrane conductances, although less remarkably than the depolarizing one. This is because the depolarization-dependent membrane conductances are in a slightly activated state at the resting membrane potential level (Takashima and Takahata, 2000).

In order to test the possibility that the variability in the shape of synaptic potentials (Fig. 2A, B) is due to location of synapses on different dendritic terminals having different electrotonic distances to the segment, we conducted a series of simulations assuming input synapses on the terminal compartment of each dendrite on the side ipsilateral to the soma.
It was found that, although the rate of rise showed variance that was comparable with that observed in physiological experiment, the half decay time was less variable in simulation than in experiment (P < 0.05; Fig. 7B). The reason of this discrepancy remains unknown, but a possibility is that the irregular narrowing of fine dendritic processes observed by electron microscopy (Kondoh and Hisada, 1986a) causes unpredictable prolongation of PSPs. Further experimental analyses and model improvements are needed to account for this discrepancy.
DISCUSSION

Although recent studies have clearly demonstrated that dendrites are richly endowed with voltage-dependent conductances, it is widely appreciated that the passive electrotonic properties of the dendritic tree provide the backbone for the electrical signaling in a nerve cell (London and Häusser, 2005; Rall et al., 1992; Segev and London, 1999). The function of dendrites as a passive integrator is particularly important for nonspiking interneurons that control the activity of postsynaptic cells by not only membrane depolarization but also hyperpolarization (Burrows and Siegler, 1976) that occurs in the voltage range where the membrane behaves passively, although their dendritic membrane is known to possess a variety of voltage-dependent conductances (Laurent, 1990, 1991; Laurent et al., 1993; Takahata et al., 1995; Takashima and Takahata, 2000). They actually receive hyperpolarizing synaptic potentials at the resting potential level (Fig. 2). In the present study, we examined the functional significance of the electrotonic structure as well as the active membrane conductances in the synaptic integration of an identified nonspiking interneuron by computer simulation using its multicompartment model.

It should be noted here that the present model is based on imperfect experimental data. Morphologically, our 3-D morphometry underestimated the membrane area by ignoring fine processes due to limitation in the practical resolution of the microscope. Physiologically, it remains unknown whether the whole neuron is uniform or not regarding its active and passive membrane properties. Taking these limitations into account, however, we made full use of the present model to simulate the synaptic activity on its dendrite since it represents the best estimate of morphological and physiological characteristics of the LDS interneuron for the time being.
Functional Significance of Active Membrane Conductances

SPATIO-TEMPORAL DISTRIBUTION OF SYNAPTIC POTENTIALS OVER DENDRITES. The depolarization-dependent outward currents such as those found in the LDS interneuron membrane (Takashima and Takahata, 2000) could function in enhancing the attenuation experienced by depolarizing synaptic inputs since these potassium currents tend to clamp the membrane potential near the resting potential level. In a system where the back propagation of action potentials into dendrites has critical relevance to the neuronal function by providing a feedback mechanism (Linden, 1999; Magee and Johnston, 1997), the voltage-dependent outward currents are thought to regulate the propagation depending on the background excitatory activities (Hoffman et al., 1997; Magee et al., 1998). Since the LDS interneuron does not generate spikes, however, the functional role of voltage-dependent conductances should be sought elsewhere.

When a hyperpolarizing synaptic potential spread from the synaptic terminal (single asterisk in Fig. 1B) to the thick transverse segment, not only the peak amplitude was intensively attenuated but also the time course was significantly expanded (Figs. 3D). These tendencies were roughly dependent on the branch length since each branch had similar diameter values. The branch length ranging from 58 to 501 µm, the attenuation factor for the peak amplitude and the expansion factor for the half decay time ranged from 2.26 to 12.93 and from 0.17 to 0.28 respectively. When a unitary depolarizing synaptic potential spread from the synaptic terminal to the thick transverse segment (Fig. 4B), the attenuation factor for the peak amplitude and the expansion factor for the half decay time ranged from 2.29 to 13.84 and from 0.19 to 0.32 respectively. Although the time course of depolarizing synaptic potentials was slightly affected by the voltage-dependent conductances (Fig. 7A), both the attenuation and expansion factors were not statistically
different between depolarizing and hyperpolarizing potentials (P > 0.05).

We also found that larger depolarizing potential spread to the synaptic terminal with similar attenuation and expansion factor values irrespective of whether the dendritic membrane from the transverse segment to the output branches was active or passive. In response to strong stimulation applied to the mechanosensory afferent bundle, the interneuron showed a large compound synaptic potential that fully activated the depolarization-dependent conductances so that the potential decayed more rapidly than in the passive condition. This characteristic signal spread to output terminals without any significant deformation (Fig. 6B). The attenuation factor was 1.12 ± 0.003 and 1.15 ± 0.004 in the active and passive condition respectively. The difference was statistically significant (P < 0.01). But, considering the fluctuation of membrane potential due to synaptic noises (Fig. 2A), we conclude that the difference is not substantial in reality. These findings, together with the results on a single synaptic potential (Fig. 6A1 inset), suggest that, as far as the spread of synaptic potentials over dendrites is concerned, the voltage-dependent potassium conductances do not have any physiological significance in the LDS interneuron.

A POSSIBILITY OF BIASED DISTRIBUTION OF ACTIVE CONDUCTANCES IN THE INTEGRATING SEGMENT. It is noted that the summation of synaptic input for activating the voltage-dependent membrane conductances to sharpen the resulting potential can be attained in the condition that only the membrane of the summing region, i.e., the transverse segment over the midline, is endowed with the conductances whereas the membrane of other regions is completely passive (Fig. 6A). On the other hand, the sharpening could be also attained when the distal membrane was endowed with the conductances while the
Synaptic integration in nonspiking interneuron

The membrane of the summing region was passive (data not shown). These findings suggest that the voltage-dependent outward conductances should not necessarily be distributed uniformly over the entire dendrite of the LDS interneuron in order to show the characteristic response as observed in reality. It has been known that active dendritic conductances show non-uniform distribution over an entire neuron (Lörizcz et al., 2002; Migliore and Shepherd, 2002): A-type potassium currents, for example, are located at higher densities in the distal part of the dendrites (Hoffman et al., 1997; Magee et al., 1998). Further study using a patch electrode is needed to test this possibility in the LDS interneuron.

Even if the active conductances should be concentrated solely to the transverse segment, the characteristic synaptic response to strong sensory stimulation cannot be realized without extensive arborization of passive dendrites connected to the segment. We found that the time course of synaptic responses at the synaptic site depends on whether the compartment having the input synapse is isolated or connected with other parts of the cell (Fig. 4C, D). The fast decay of voltage as observed in Fig. 4C is a general property of extended dendrites (Segev and London, 1999). The slower responses shown in Fig. 4D are more similar to actually observed ones. It thus follows that even if the LDS interneuron were of a single isopotential structure, its synaptic responses to increasing sensory stimuli would be similar to those actually observed (Fig. 2C, 5A) provided that the conductance increase caused by the increase in stimulus intensity is large enough. This situation, however, might be difficult to be attained in natural conditions since the synaptic input is summed up nonlinearly (Koch, 1999; Shepherd, 2004). On the other hand, the synaptic responses of the LDS interneuron to increasing sensory stimuli would not be the same as those actually observed (Figs. 2C, 5A) if the input synapse were restricted to a
single terminal and an increase in the sensory stimulus were realized by an increase in the synaptic conductance. The extensive arborization of dendrites that receive synaptic input at many sites, as evinced by anatomical observation (Fig. 1A) and therefore assumed in the present modeling (Fig. 1B), is thus of functional significance in that the time course of the potential summed up in a single region is sculpted by the membrane conductances there to be much sharper than ever (Figs. 2C, 5A).

*Functional Significance of the Electrotonic Structure of Dendrites*

**ELECTROTONIC STRUCTURE.** The synaptic potential, once reaching the thick segment near the midline, invaded other dendritic branches without any noticeable attenuation in the peak amplitude nor expansion in the half decay time (Figs. 3B, 4A) irrespective of its polarity and initial peak amplitude. This finding suggested that the thick segment of nonspiking interneurons would serve as the integration site for a variety of synaptic inputs originating from many dendrites: the membrane potential change in the segment produced as the result of addition and subtraction among synaptic potentials determines the peak amplitude and the time course of synaptic outputs from the dendritic terminals. In arthropod motoneurons, in which input synapses are also distributed over fine branches (Kondoh and Hisada, 1986b; Watson and Burrows, 1982), the thick dendritic segment is thought to function in integrating synaptic inputs to determine the spike output from the cell (Evoy, 1977; Gwilliams and Burrows, 1980). Although nonspiking interneurons do not generate spikes, the functional role of thick dendritic segments of these interneurons and motoneurons appears to be common.

It is clear from the present results (Fig. 3D) that, when the input and output synapses are present in different dendritic branches, the most important factor determining the
Synaptic output is how electronically far the input synapse is located from the thick segment, wherever the output synapse is located. The spread of synaptic potentials toward the thick segment depends on the branch morphology and membrane characteristics (Rall, 1981; Rall et al., 1992). Thus when the input synapse is located more distally over a long, thin branch, the synaptic potential tends to be more attenuated in the peak amplitude and more smoothed in the time course during its spread over the dendritic branch (Fig. 3D). When the synaptic input occurs sequentially, the synaptic potential makes temporal summation with other ones to develop a sustained hyperpolarization in the thick segment (Fig. 3C). This tendency was more remarkable when the input synapse was located in a more distal site of the dendrite. Functional significance of such a sustained hyperpolarization is not clear for the LDS interneuron. However, for those nonspiking interneurons involved in premotor circuits controlling the activity of motor neurons, such a sustained hyperpolarization could produce a sustained activation of motor neurons for gating their spike activity in cooperation with other excitatory inputs (Murayama and Takahata, 1998; Takahata and Hisada, 1986).

LOCAL PROCESSING. Nonlinear interaction between synaptic inputs in a restricted dendritic region where they colocalize has been well appreciated in spiking neurons. Neighboring synapses affect each other by changing the membrane potential of each synaptic site and hence the driving force for each input (Koch, 1999; Rall et al., 1967). Extensive arborization of dendrites enables the spatial separation of inputs to minimize their interaction. In such a condition, each dendrite can carry out computation independently of each other, performing parallel processing of synaptic inputs simultaneously (London and Häusser, 2005). The results of local computation in different dendritic regions are further
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integrated at the spike initiating site. Thus, in a spiking neuron, each dendritic region can be regarded as a pre-processor for the spike initiating site that works as the main processor of all synaptic inputs to determine whether a spike is generated or not as the sole output of the neuron, except the case of dendrodendritic synapses reported in some spiking neurons (Hamos et al., 1985; Rall et al., 1966; Woolf et al., 1991).

Nonspiking interneurons, on the other hand, do not generate spikes. Their synaptic output, i.e., the amount of transmitter release, is regulated directly by their membrane potential (Burrows and Siegler, 1976, 1978) while the output synapses are distributed all over the dendrites, mostly on peripheral thin processes (Kondoh and Hisada, 1986b; Watson and Burrows, 1988). In the LDS interneuron that extends dendrites on both sides of the ganglion, the input and output synapses are distributed exclusively on fine processes (Kondoh and Hisada; 1986a): many input and output synapses are intermingled on the contralateral dendrites and, though much less densely, on the ipsilateral dendrites as the dendrodendritic synapses in the vertebrate brain (olfactory bulb, Rall et al., 1966; cerebral cortex, Häusser et al., 2000; Segev and London, 2000). Thus, in a nonspiking interneuron, each dendritic region can work as an independent processor that integrates local inputs to form a local output (Pearson, 1976; Wilson and Phillips, 1983). In order to test this possibility, we need detailed information on the input-output relationship in a well-characterized neuronal circuit at the dendrite level rather than the cell level. However, no such information is available presently. In this study, we calculated how the synaptic potential, either depolarizing or hyperpolarizing, was attenuated as it spread from the synaptic site to other regions to study the potential effect of one local synaptic activity upon input and output synapses on other branches. Our calculation revealed that a hyperpolarizing synaptic potential originating at one terminal attenuated by a factor of 5.7
and 6.1 when it spread to other terminals of ipsilateral and contralateral branches respectively (Fig. 3B). Both small and large depolarizing synaptic potentials showed comparable attenuation factors (Fig. 4A). Evaluation of these figures is difficult without information on the location of input and output synapses as well as the functional roles of each local circuit. Our present conclusion is that dendritic branches are functionally well isolated from one another, yet the possibility remaining that the membrane potential change in one process can still affect others if the change is enhanced by temporal summation of synaptic currents or if the attenuation factor is reduced by alteration in the background synaptic activity (Bernander et al., 1991; Rapp et al., 1992) or, possibly, the action of neuromodulatory factors (Brown, 1988; Reuter, 1983).

In this study, we made calculations for an input synapse only on the soma side. We think, however, that the conclusion holds for the case where the input synapse is located on the opposite side since we quantitatively demonstrated that the electrotonic structure of dendrites is similar on both sides of the LDS interneuron (Hikosaka et al., 1996).

Functional Implications for the LDS Interneuron

Voltage-dependent membrane conductances were found to exert no significant effect on the spatial distribution of synaptic potentials on dendrites of the LDS interneuron (Fig. 6). Since all three types of potassium conductances are mostly activated upon depolarization from the resting potential (Takashima and Takahata, 2000), depolarizing synaptic potentials were more affected by them than hyperpolarizing ones when the interneuron was at rest (Fig. 7). The effect of these conductances was most remarkable when a large compound depolarizing response was evoked by electrical stimulation of mechanosensory afferents (Fig. 2C). The response consisted of a fast spike-like component
followed by a slower potential which showed various time courses (Figs. 2C, 5A, 5D1-2; Krenz and Reichert, 1985; Reichert et al., 1983). They shared a common characteristic that the spike-like component appeared only when the peak amplitude reached about 20 mV or more from the resting potential. This observation is consistent with the voltage kinetics of potassium conductances of the LDS interneuron (Takashima and Takahata, 2000). If the conductances were completely absent in the membrane, the same synaptic input caused a larger slow response without the initial fast component (Fig. 5C). When the large input came sequentially, the slow synaptic response made temporal summation with each other to obscure individual input in the passive-membrane model (Fig. 5F). The temporal resolution in processing successive synaptic inputs becomes thus significantly high if the membrane possesses voltage-dependent conductances. Even when the synaptic input was not very large, the effect of active membrane properties could be observed in the temporal summation of successive responses (Fig. 5E).

The LDS interneuron mediates the lateral inhibition in the tailfan mechanosensory system to enhance its directional sensitivity (Krenz and Reichert, 1985; Reichert et al., 1982). The excitatory synaptic input to the LDS interneuron from mechanosensory afferents on the side ipsilateral to its cell body is transmitted through the transverse segment to dendrites on the other side where the interneuron exerts an inhibitory postsynaptic effect on ascending interneurons (Reichert et al., 1983). When the water current stimulus occurs in rapid succession, it would be advantageous for the LDS interneuron to transmit information from one side to the other with the temporal resolution high enough for discriminating individual input, although this possibility is to be tested experimentally. The voltage-dependent membrane conductances thus can have a crucial significance for the LDS interneuron by keeping the synaptic responses temporally tight.
ACKNOWLEDGMENTS

We thank M. Niwa and H. Nakamura for helpful assistance in physiological experiments.
GRANTS

This work was supported in part by Research Fellowships for Young Scientists (AT, RH) and Grants-in-Aid (MT) from the Japan Society for the Promotion of Science (14340260, 17370024).
Figure 1 A Double staining of the LDS interneuron (yellow, indicated by an arrowhead in light grey) and sensory afferents (orange, arrowhead in dark grey). Sensory afferents appear to make contact with the interneuron at many of its dendritic branches on the side ipsilateral to the cell body. B Reconstructed projection image (grey) of an LDS interneuron based on 3-dimensional morphometry to show the site of microelectrode impalement into the cell during experiment (arrowhead) and dendritic regions where synaptic connections with affrents were assumed in simulation (black). Other symbols are explained in the legend of related figures below.

Figure 2 Physiological characterisitics of the LDS interneuron. A Spontaneous synaptic activity involving depolarizing and hyperpolarizing discrete potentials. Two representative traces are shown above and other two below in a slower time scale. B Variance in the shape of synaptic potentials. Depolarizing (open circle) and hyperpolarizing (filled circle) potentials are characterized by the rate of rise (abscissa) and the half decay time (ordinate). C Compound synaptic potentials in response to electrical stimulation of the sensory nerve bundle at increasing intensity. D Recorded voltage responses to intracellularly injected current at the resting potential level. The microelectrode impaled the interneuron on the midline. The arrowhead in this and following panels indicate the transient peaks caused by rapid repolarization due to potassium conductances. E Calculated voltage responses to current injection in the same midline region. F Recorded voltage responses to current injection in the distal dendritic region. The variability of the transients depended not on the recorded region but on the preparation as described in the text. D and F were published previously (Takashima and Takahata, 2000 and Takahashi et al., 1995, respectively), but were included here for comparison.
Figure 3  Electrotonic spread of hyperpolarizing synaptic potentials over dendrites. A Recorded (black solid) and calculated responses (color dotted) in the b region shown in Fig. 1B elicted by a hyperpolarizing step current injected into the same region. The membrane area of the soma was assumed to be 5 times (blue) and 20 times (red) larger than that of a simple ellipsoid as approximated in the present 3-D morphometry. The residuals for each approximation are shown at the top of the panel. B Calculated hyperpolarizing potentials in representative regions of the LDS interneuron. Assuming a synapse with an afferent fiber on a terminal compartment (shown with * in Fig. 1B), voltage responses were calculated for the synaptic region (*), four dendritic regions (a-d in Fig. 1B) and the soma. The cell was assumed to be at the resting potential level initially. The inset shows the attenuation factors experienced by the synaptic potential originated in the * region spreading to other branch terminals of ipsilateral (gray bar) and contralateral (black bar) dendrites. C Temporal summation of synaptic potentials. Presynaptic spikes were assumed to reach the terminal repetitively at 100 Hz. D Voltage attenuation in the course of dendritic spread from the synaptic terminal on the longest (blue, ** in Fig. 1B) and shortest (red, *** ) branch to the transverse segment. Each response in the transverse segment was normalized to the original one in the synaptic region.

Figure 4  Dendritic spread of depolarizing synaptic potentials. A Synapse with an afferent was assumed to be located on a terminal compartment (shown with * in Fig. 1B). A Calculated depolarizing potentials in the synaptic (*) and four dendritic regions (a-d in Fig. 1B) as well as the soma, each drawn by a black solid line. The cell was assumed to be at the resting potential level initially. The inset shows the attenuation factors experienced by
the synaptic potential originated in the * region spreading to other branch terminals of ipsilateral (grey bar) and contralateral (black bar) dendrites. The total potassium conductance activated by the synaptic potential in each region is drawn by a blue line. B Voltage attenuation of a single depolarizing synaptic potential during its spread from the synaptic terminal on the longest (green, shown with ** in Fig. 1B) and shortest (red, ***) branch to the transverse segment. Each response in the transverse segment was normalized to the original one in the synaptic region. C Voltage responses of the terminal compartment connected with other parts of the model to increasing conductance changes. D Voltage responses of the terminal compartment isolated from other parts of the model to increasing conductance changes. The insets in C and D show connection of compartments (grey rectangles) near the synaptic terminal (black rectangle).

**Figure 5** Recorded and calculated compound synaptic responses to increasing sensory stimulation. A Recorded responses. They are most typical for the interneuron and were reported previously (Takashima and Takahata, 2000). They are reprinted here for comparison with the results of simulation. B Calculated responses in the model with depolarization-dependent conductances implemented to the membrane of all compartments. The increase in stimulus intensity caused more synapses to be activated in the model. Location of synapses is shown in Fig. 1B. C Calculated responses in the model with no depolarization-dependent conductance implemented. D1-2 Recorded responses in different preparations. E Temporal summation on active (grey) and passive (black) membranes at a weak stimulus intensity. The depolarization-dependent conductances were not fully activated. F Temporal summation at a stronger intensity when conductances were fully activated.
Figure 6 Effects of non-uniform distribution of active conductances on synaptic integration and current spread over dendrites. A Compound synaptic potentials in response to increasing sensory stimulation in a uniform (A1) and a non-uniform model (A2) where active conductances were distributed only on the membrane of the transverse segment. The inset to A1 shows single depolarizing synaptic potentials produced at a branch terminal (*) and calculated for the region \( a \) (Fig. 1B) in the uniform (red) and non-uniform (blue) models as well as in the passive model (black). The results in three models are normalized to the potential change at the synaptic site respectively. Note that all three traces are completely overlapped each other until the late falling phase at the synaptic site and until the early falling phase in the region \( a \). B Attenuation of the compound synaptic potential when it spread from the transverse segment to an output terminal \( d \) in Fig. 1B over uniform (B1) and non-uniform (B2) membranes. The compound synaptic potential was evoked by simultaneous activation of all synapses assumed on the terminal compartments of dendritic branches on the soma side (shown with black in Fig. 1B). The attenuation factors for all 17 output terminals are compared in the inset of B1 between uniform (gray) and non-uniform (black) membranes.

Figure 7 Simulation of discrete synaptic potentials caused by the spontaneous spike activity of sensory afferents. A Calculated depolarizing and hyperpolarizing potentials fit to the recorded data. The calculation was carried out on the condition that a single synapse was located in one of the terminal compartments on the side ipsilateral to the cell body. The conductance change at the synapse was adjusted to yield a comparable voltage change in the recorded region. The red and blue lines were obtained using active and passive
membranes respectively. **B** Distribution of shape parameters for the recorded (open circles) and calculated (filled circles) depolarizing synaptic potentials. Abscissa: the rate of rise, ordinate: the half decay time. The ordinate is expanded in the inset.
Table 1 Parameters and their values used in the present model. Functional meanings of the parameter signs are fully described in the text.

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*Er, reversal potential; $m$, voltage-dependent activation gate; $h$, voltage-dependent inactivation gate.
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