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

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Takashima, Akira, Ryou Hikosaka, and Masakazu Takahata. Functional significance of passive and active dendritic properties in the synaptic integration by an identified nonspiking interneuron of crayfish. J Neurophysiol 96: 3157–3169, 2006. First published August 16, 2006; doi:10.1152/jn.00680.2006. 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 multicompartiment model based on electrophysiological measurements and three-dimensional morphometry. At the resting potential level, the attenuation factor ($V_s$) of a unitary synaptic potential in the course of its spread from a dendritic terminal ($V_t$) to other terminals ($V_v$) ranged from 4.42 to 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 2004; Marder and Bucher 2001) and motor control (Hama and Takahata 2005; Murayama and Takahata 1998) systems of arthropods. For example, the LDS (locally directionally selective) 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). Thus 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 also be 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; 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. Thus the most important event 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 spatiotemporal distribution of synaptic potentials over the whole dendrite is important for understanding their synaptic integration capabilities because 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 LDS interneurons have three distinctive active conductances: a sustained component and two kinds of transient components (Takashima and Takahata 1998).
kept in a refrigerator for 4 h. The preparation was fixed in 10% 
nerve fibers, the cut end of the second or third root of the terminal 
electrophysiological experiments are described in a previous report. 
the damage arising from electrode penetration to a minimum. Details 
transverse segment of the interneuron on or near the midline to keep 
yellow in 1 M LiCl. Intracellular recording was made from the thick 
electrophysiological experiments were carried out with a single bo-
of the body and pinned to a silicone elastomer-lined chamber with its 
including the terminal abdominal ganglion was isolated from the rest 
TABLE 1. Parameters and their values used in the present model

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>$E_r$, mV</th>
<th>Gating Variable</th>
<th>$p$</th>
<th>$q$</th>
<th>$A$</th>
<th>$B$</th>
<th>$C$</th>
<th>$D$</th>
<th>$E$</th>
<th>$F$</th>
<th>$G$</th>
<th>$H$</th>
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</thead>
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<tr>
<td>Sustained</td>
<td>$g_s$</td>
<td>$-70$</td>
<td>$m$</td>
<td>4</td>
<td>—</td>
<td>0.46</td>
<td>1</td>
<td>28</td>
<td>-11</td>
<td>0.003</td>
<td>0</td>
<td>0</td>
<td>-15</td>
</tr>
<tr>
<td>Transient1</td>
<td>$g_{s1}$</td>
<td>$-70$</td>
<td>$m$</td>
<td>4</td>
<td>—</td>
<td>2.438</td>
<td>1</td>
<td>33</td>
<td>-6</td>
<td>0.0062</td>
<td>0</td>
<td>0</td>
<td>-21</td>
</tr>
<tr>
<td>Transient2</td>
<td>$g_{s2}$</td>
<td>$-70$</td>
<td>$h$</td>
<td>—</td>
<td>1</td>
<td>0.121</td>
<td>0</td>
<td>0</td>
<td>-19</td>
<td>2.6</td>
<td>1</td>
<td>0</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$h$</td>
<td>1</td>
<td>—</td>
<td>0.049</td>
<td>1</td>
<td>31</td>
<td>-5</td>
<td>0.02</td>
<td>0</td>
<td>0</td>
<td>-42</td>
</tr>
</tbody>
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$E_r$, reversal potential; $m$, voltage-dependent activation gate; $h$, voltage-dependent inactivation gate. Functional meanings of the parameter signs are fully described in the text.

Multicompartamental 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 <0.1A, where $\lambda$ is the length constant, so that each compartment could be regarded as isopotential. $\lambda$ was obtained using the following relationship

$$\lambda = \frac{DR_m}{4R_m}$$

where $D$ is the dendritic diameter obtained by morphological measurement, $R_m$ is the axoplasm resistivity assumed to be 0.06 k$\Omega$cm, and $R_m$, 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 multicompartamental model for the present study, the experimentally obtained value for $\tau_m$ was 25.8 ms, 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 ($g_{s1}$, $g_{s2}$) 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 2000). Experimental analyses suggested that all three kinds of voltage-dependent conductances are carried by potassium ions.

Thus the whole ionic current flowing through the membrane including the leak current is expressed as

$$I_m = g_s(V - E_k) + g_{s1}(V - E_k) + g_{s2}(V - E_k) + g_{leak}(V - E_{leak})$$

in which $E_k$ and $E_{leak}$ are the equilibrium potentials for outward and leak current, respectively. $E_k$ was -70 mV according to the experimental data. $E_{leak}$ was assumed to be the same as the resting potential. The voltage- and time-dependent parameters for the three potassium conductances ($g_s$, $g_{s1}$, and $g_{s2}$) 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

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

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

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

\[
g = \bar{g}_n m^h (V)
\]

where \(g\) is the membrane conductance and \(\bar{g}\) is the maximal membrane conductance for the potassium ion. The values for \(g\) and \(\bar{g}\) 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

\[
dm/dt = \alpha_m(1 - m) - \beta_m m
\]

in which \(\alpha_m\) and \(\beta_m\) are voltage-dependent rate constants for transition of the gate to permissive and nonpermissive 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 by 1.2 to compensate for the shrinkage arising from fixation and clearing procedures that followed fixation (Hikosaka et al. 1996). It was found, however, even after this shrinkage compensation, that 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 a result of the current procedure that each dendritic branch was approximated by one or more cylindrical compartments. It was also likely that those processes with diameters <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 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 fourfold larger than the estimated value in a cylinder or sphere having a volume comparable to that of the soma (Watson and Burrows 1983). We therefore limited the soma surface compensation factor to the order of four to six, 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 areas were assumed to be fivefold and 1.27-fold 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) 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 \(g_{syn}(V_i - V_{syn})\) to the equation for \(I_m\) (Eq. 1); \(g_{syn}\) is the synaptic conductance and \(V_{syn}\) is the reversal potential assumed to be 0 mV for the excitatory input and ~70 mV for the inhibitory input.

**Fig. 1.** A: double staining of the locally directionally selective (LDS) interneuron (yellow, indicated by an arrowhead in light gray) and sensory afferents (orange, arrowhead in dark gray). 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 (gray) of an LDS interneuron based on 3-dimensional (3-D) morphometry to show the site of microelectrode impalement into the cell during experiment (arrowhead) and dendritic regions where synaptic connections with afferents were assumed in simulation (black). Other symbols are explained in the legend of related figures below.
can be discriminated individually. It can be seen in the record. They are separated from each other in most the branches on the same side. When recorded in the transverse ventromedially in the middle of the ganglion and connected to the dorsal half of the ganglion (Fig. 1A). The cell body is located 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 ventromedially 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 ± 0.04 mV/ms (mean ± SE) and the half-decay time was 15.70 ± 0.93 ms in the depolarizing potentials (n = 27), whereas they were 0.15 ± 0.01 mV/ms and 23.60 ± 1.90 ms 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 ≥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 >10 mV from the resting potential (Fig. 2D). These findings indicate that, because of 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 as a result of membrane damages caused by microelectrode penetration and of deterioration of the preparation. They were clearly seen in another experiment (Fig. 2F).

Electrotonic spread of hyperpolarizing potentials over dendrites

Previous studies 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 region b shown in Fig. 1B. After appropriate modification of structural data (see METHODS), 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 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 because those values were unrealistic (see METHODS). 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 fivefold larger.

Using this passive model of the LDS interneuron, we analyzed the spatiotemporal 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 effectively reduce it (Takahata et al. 1995), suggesting that the directional sensitivity of the tailfan mechanosensory system is more enhanced by this inhibitory pathway. The hyperpolarizing synaptic activity is thus crucially significant in the functioning of the LDS interneuron. We studied how a discrete hyperpolarizing potential (Fig. 2A) spread over dendrites after being 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 five representative regions of the LDS interneuron including its cell body (a–d in Fig. 1B). Not only was the peak amplitude considerably reduced but the time course was also significantly expanded when the synaptic potential spread to 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 and 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 because 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 ($V_s$) to another terminal ($V_i$) as $V_s/V_i$. It was $5.74 \pm 0.09$ ($n = 29$; mean $\pm$ SE) and $6.10 \pm 0.01$ ($n = 17$) for ipsilateral and contralateral branches, respectively. The expansion factor for the half-decay time ($T_t$) during the voltage spread from the synaptic site ($T_s$) to another terminal ($T_i$) was defined as $T_s/T_i$ in this study. It was $0.21 \pm 0.003$ and $0.20 \pm 0.001$ (mean $\pm$ SE) for ipsilateral and contralateral branches, respectively. The differences in the attenuation factor were statistically not significant (Student’s
We found that the excitatory synaptic potential behaved in a way similar to that of 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 the time course was significantly expanded in 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 and d). When a depolarizing synaptic potential spread from the synaptic region at one dendritic terminal (V_s) to other terminals (V_j), the peak attenuation factor (V/V_s) 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 (V_s) to the transverse segment crossing the midline (V_a), the peak attenuation factor (V/V_s) 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 depolarization 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 a lesser 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 that 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 shoulderlike slow decay (Fig. 5A). To reproduce this characteristic response pattern, we had to assume that many peripheral compartments simultaneously received excitatory synaptic input. Assuming the synaptic input on only one terminal compartment, the membrane potential change arising from synaptic activity was very small when recorded in the transverse segment (<1 mV) even though...
the conductance change was adjusted so as to produce a large synaptic potential at the terminal (Fig. 4, A and 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 increase 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 of natural responses (Figs. 2C and 5A). Because the afferent fibers appear to make contact with the LDS interneuron at many sites (Fig. 1A), we consider the present assumption reasonable, although 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 be reproduced in simulation only 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; both the time width of the peak and the voltage level at which the shoulder in the falling phase appeared varied from preparation to preparation (Figs. 2C and 5, A and 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 and 5, A, D1, and D2) is caused by partial failure of depolarization-dependent membrane conductances because of 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 to lock 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 nonuniform 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 depolariza-
tion-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 nonuniform 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 nonuniform 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 was 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 was 1.12 ± 0.003 ($n = 17$), ranging from 1.08 to 1.14, whereas the expansion factor was 0.89 ± 0.01 ($n = 17$), ranging from 0.82 to 0.90. Those values were 1.15 ± 0.004 ($n = 17$), ranging from 1.10 to 1.17, and 0.84 ± 0.01 ($n = 17$), ranging from 0.82 to 0.90, respectively, in the nonuniform model (Fig. 6B2) in which only the membrane of the transverse segment behaved actively. Both values showed statistical differences between the uniform and nonuniform models ($P < 0.01$). When considering the fluctuation of membrane potential arising from 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 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 (Fig. 6B) and the midline region (A) in the uniform and nonuniform 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

Because 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.

**FIG. 6.** Effects of nonuniform 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 nonuniform model (A2) where active conductances were distributed only on the membrane of the transverse segment. Inset, A1: single depolarizing synaptic potentials produced at a branch terminal (•) and calculated for region a (Fig. 1B) in the uniform (red) and nonuniform (blue) models as well as in the passive model (black). Results in 3 models are normalized to the potential change at the synaptic site, remaining under the threshold for activation of depolarization-dependent conductances, but became steeply deformed by them when it exceeded the threshold. The total conductance change was the same as the experimentally obtained value in both the uniform and nonuniform models.

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 nonuniform (B2) membranes. 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). Attenuation factors for all 17 output terminals are compared in the inset of B1 between uniform (gray) and nonuniform (black) membranes.
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).

To test the possibility that the variability in the shape of synaptic potentials (Fig. 2, A and B) is ascribed 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 for 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 postsynaptic potentials (PSPs). Further experimental analyses and model improvements are needed to account for this discrepancy.

**DISCUSSION**

Although recent studies 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 both the functional significance of the electrotonic structure and 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 three-dimensional morphometry underestimated the membrane area by ignoring fine processes resulting from limitation in the practical resolution of the microscope. Physiologically, it remains unknown whether the whole neuron is uniform with respect to its active and passive membrane properties. By taking these limitations into account, however, we made full use of the present model to simulate the synaptic activity on its dendrite because it represents the best estimate of morphological and physiological characteristics of the LDS interneuron for the time being.

**Functional significance of active membrane conductances**

**SPATIOTEMPORAL 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 because 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). Because 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 was the peak amplitude extensively attenuated but also the time course was significantly expanded (Figs. 3D). These tendencies were roughly dependent on the branch length because each branch had similar diameter values. The branch length ranged from 58 to 501 μm and 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 potentials fit to the recorded data. 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. Conductance change at the synapse was adjusted to yield a comparable voltage change in the recorded region. 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. Ordinate is expanded in the inset.
synaptic potentials was slightly affected by the voltage-dependent conductances (Fig. 7A), both the 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 $1.12 \pm 0.003$ and $1.15 \pm 0.004$ in the active and passive conditions, respectively. The difference was statistically significant ($P < 0.01$). By considering the fluctuation of membrane potential arising from synaptic noises (Fig. 2A), however, we conclude that the difference is not substantial in reality. These findings, together with the results on a single synaptic potential (Fig. 6A, inset), suggest that, with respect to the spread of synaptic potentials over dendrites, 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 inputs to determine the spike output from the cell thick dendritic segment is thought to function in integrating sensory stimuli would be similar to those actually observed (Figs. 2C and 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 because 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 and 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 and 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 and 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 co-localize has been well appreciated in spiking neurons. Neighboring synapses affect each other by changing the membrane potential of each synaptic site and thus 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 integrated at the spike-initiating site. Thus in a spiking neuron, each dendritic region can be regarded as a preprocessor for the spike-initiating site that works as the main processor of all synaptic inputs to determine whether a spike is generated as the sole output of the neuron, except in 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), whereas 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, although 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). 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 on input and output synapses on other branches. Our calculation revealed that a hyperpolarizing synaptic potential originating at one terminal attenuated by factors 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 remains 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. We think, however, that the conclusion holds for the case where the input synapse is located on the opposite side because 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). Because all three types of potassium conductances are mostly activated on 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 spikelike component followed by a slower potential that showed various time courses (Figs. 2C and 5, A, D1, and D2; Krenz and Reichert 1985; Reichert et al. 1983). They shared a common characteristic that the spikelike component appeared only when the peak amplitude reached about ±20 mV 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 thus becomes 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.
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