Dendritic Calcium Channels and Their Activation by Synaptic Signals in Auditory Coincidence Detector Neurons

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METHODS

Slice preparations

Experiments were done with the approval of IACUC for Oregon Health and Science University. The 230- to 250-μm-thick brain stem slices were prepared from chick embryos 14–18 days of age (E14–E18) according to the protocols described previously (Zhang and Trussell 1994). The oxygenated and cold (–4°C) saline for dissection of the slices contained the following (in mM): 140 NaCl, 5 KCl, 3 CaCl₂, 1 MgCl₂, 10 HEPES, and 15 d-glucose, pH adjusted to 7.4 with NaOH. The slices were incubated in the same saline solution warmed to 30°C for a minimum of 1 h before recordings or imaging.

Electrophysiological recordings

Slices were bathed in an oxygenated saline solution that for voltage-clamp Ca²⁺ channel recordings contained (in mM): 5 NaCl, 120 TEA-Cl, 10 CsCl, 1 4-aminopyridine, 3 CaCl₂, 1 MgCl₂, 10 HEPES, and 15 d-glucose and (in μM) 2 TTX, 25 DNQX, or NBQX, 10 SR-95531, and 1 strychnine, pH adjusted to 7.4 with CsOH. For current-clamp recording, the bath solution contained in (mM) 140 NaCl, 5 KCl, 3 CaCl₂, 1 MgCl₂, 10 HEPES, and 15 d-glucose, pH adjusted to 7.4 with NaOH. In imaging experiments, the bath solution was the same as that used in slice preparation. Internal solutions for voltage-clamp recordings contained (in mM) 106 CsMeSO₄, 4.5 MgCl₂, 14 Tris-phosphocreatine, 4 MgATP, 0.3 Tris-GTP, 10 EGTA, 10 HEPES, and 20 TEA-Cl, osmolarity adjusted to 280 mOs/m with sucrose, pH adjusted to 7.3 with CsOH. For current-clamp recordings during two-photon image acquisition, the internal solutions contained (in mM) 113 K-Gluconate, 9 HEPES, 4.5 MgCl₂, 0.1 EGTA, 14 Tris-phosphocreatine, 4 Na₂-ATP, and 0.3 Tris-GTP; osmolarity

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brought to −290 mOsm with sucrose, pH adjusted to 7.25 with KOH, plus indicator dyes as described below. Whole cell patch recordings were made with an Axon Multiclamp 700A (Molecular Devices) for voltage clamp or a Dagan BCV700A for current clamp and were performed at room temperature (22–24°C) except as noted. The recording pipettes, pulled from thin-wall borosilicate glasses (World Precision Instruments, Sarasota, FL), typically had a series resistance of 2.5–5 MΩ before 70–80% compensation. Reported voltages are corrected for a junction potential of −11 mV in voltage- and current-clamp experiments. A P/4 subtraction of leak and capacitative currents was applied using pClamp 9.2 (Molecular Devices). The electrophysiological data were filtered at 8 kHz, digitized with Digidata 1200 or 1322A, and acquired through pClamp 9 (Molecular Devices). The recordings were analyzed using Axograph 4 (Molecular Devices) and Igor 5.0 (WaveMetrics, Lake Oswego, OR).

On initial patch rupture in our low Na⁺ bath solution, spikes (presumably Ca²⁺ spikes) escaping from voltage clamp were readily apparent on depolarization. Over several minutes, as pipette solution entered the cell, these disappeared, and final current-voltage relations were without evidence of discontinuities in current that indicate poor clamp. We cannot exclude that more distal dendrites may have escaped voltage clamp, but if so, they did not generate sufficient current to distort the whole cell traces.

### Imaging

**CONVENTIONAL FLUORESCENCE.** Cells were filled with 100 μM Fluo-4 \([K_4 (Ca^{2+}) = 345 \text{ nM}]\) or Fluo-5F \([K_4 (Ca^{2+}) = 2.3 \mu \text{M}]\) through the recording electrode containing the K-glucionate–based solution. Images were acquired using an AxioCam HSM (Zeiss) and Starlion software (Triple I) at speeds from 20 to 45 Hz. Data were analyzed using open source WICF ImageJ (http://www.uzhresearch.ca/facilities/wcif/image/installing_image.htm) and Microsoft Excel. Absolute fluorescence values from three image files acquired sequentially under identical conditions were obtained in ImageJ and averaged in Excel. Fluorescence values were background subtracted (subtraction of average intensity from an off-cell region) and reported as the change in fluorescence over the initial fluorescence value at a given region of interest (ROI).

**TWO-PHOTON IMAGING.** EGTA was omitted from the internal solution (because the indicator dye buffers Ca²⁺) and replaced with 200 μM Fluo-5F and 20 μM Alexa Fluor 594 hydrazide (Molecular Probes, Eugene, OR). This lower affinity indicator dye was used to reduce dye saturation (Yasuda et al. 2004). Slices were maintained at 32–33°C. A Ti:sapphire pulsed laser (Chameleon Ultra I, Coherent, Auburn, CA) tuned to 810 nm powered a Prairie Technologies Ultima two-photon imaging system (Middleton, WI). Laser intensity was controlled with an electro-optical modulator (350–80 LA, Conoptics, Danbury, CT). Cells were imaged with a ×60, 0.9 NA objective (Olympus, Melville, NY). Epi- and transfluorescence signals were split into red (R; Alexa 594) and green (G; Fluo-5F) channels using dichroic mirrors and band-pass filters (epi: 575 DCXR, HQ525/70, HQ607/45; trans: T560LPXR, ET510/80, ET620/60; Chroma, Rockingham, VT) and focused on multilayer photomultiplier tubes (R9110, Hamamatsu, Bridgewater, NJ). Electrical stimuli (30–90 V, 100–200 ms duration) were applied through bipolar electrodes fabricated from theta glass pipettes (Sutter Instruments, Novato, CA). Stimulus electrodes were placed adjacent to the most dorsal region of the imaged cells’ dendrites. Dendrites were imaged in linescan mode (446 Hz), orthogonal to their major axis. Twenty to 40 events were averaged per site [6-s interstimulus interval (ISI)], and expressed as 100 × (G/R)/(G/R)_{max}, where (G/R)_{max} is maximal fluorescence in saturating (2 mM) calcium (Yasuda et al. 2004). A total of 93.7% of all calcium transients [single excitatory postsynaptic potentials (EPSPs): 25/25; EPSP train: 46/48; single spikes: 107/117] were <20% of (G/R)_{max} and thus within the indicator’s linear range (Yasuda et al. 2004). Calcium imaging data were analyzed using open source ImageJ and Igor Pro. Absolute fluorescence values from sequential linescans acquired under identical conditions were obtained using Plot Profile in ImageJ and averaged in Igor.

### Statistics

Student’s t-test was performed where indicated using Excel. All of the data in the figures are shown as means ± SE. In the imaging data, N is given as numbers of cells and total number of sites scanned. Means in imaging data reported as means of all scanned sites.

### Reagents

APV, 2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[f]quinoxaline (NBQX), and SR 95531 were from Tocris Bioscience (Bristol, UK). TTX was from Alomone Labs (Jerusalem, Israel). Fluo-4 and Fluo-5F were from Molecular Probes (Eugene, OR). All other salts and chemicals were from Sigma-Aldrich (St. Louis, MO).

### Results

**Ca²⁺ currents in NL**

Voltage-clamp pulses, delivered in 5-mV increments from a holding potential of −81 mV, elicited two types of inward currents. Beginning at about −65 mV, a slowly rising current was activated that inactivated over 20–30 ms (Fig. 1B, arrow). When the voltage neared −40 mV, a larger, rapidly rising current turned on that showed comparatively little inactivation over the 50-ms duration of the pulse (Fig. 1, B and D). Inward currents were relatively large, with a peak value at −11 mV of −2.67 ± 0.45 nA (n = 5) and were observed in embryos between ages E14 and E18. These two phases of current activation led to a “bump” in the peak IV relation, suggesting the presence of two types of channel (Fig. 1C, arrow). Currents activated from −61 mV lacked both the fast inactivating component and the bump in the IV relation (Fig. 1, B and E). The presence of these currents in TTX and low extracellular [Na⁺], along with the apparently positive reversal potential (Fig. 1, C and E), suggests that these are both Ca²⁺ currents but with different voltage sensitivities and kinetics. For purposes of this analysis, we will refer to these as high voltage– and low voltage–activated channels (HVA and LVA, respectively).

Subtraction of the current waveforms obtained using the two holding potentials (−61 and −81 mV) allowed us to resolve biophysical characteristics of the LVA, as shown in Fig. 2. This current began activating at −65 mV and reached peak current just positive to −40 mV (Figs. 1B and 2B). Measurements of current at more positive potentials was complicated by activation of HVA. Inactivation was strongly voltage dependent, with a time constant of 34.2 ± 4.0 ms at −51 mV, dropping to 13.1 ± 1.3 ms at −16 mV (Fig. 2D). The voltage dependence of steady-state inactivation was assessed by delivering depolarizing steps from −101 mV to inactivate channels, followed by a test step to −51 mV. Inactivation began with conditioning steps to −81 mV and was nearly complete at −40 mV, with a V_{1/2} of inactivation of −64.1 ± 0.5 mV (n = 4).

The voltage sensitivity and kinetics of inactivation (Fig. 2D) strongly suggest that LVA current is generated by T-type Ca²⁺ channels (Perez-Reyes 2003).
As with T-type channels in other cell types, LVA current activation was relatively slow, raising the question of whether these channels could be activated by fast synaptic signals or action potentials. Because the experiments described above were conducted at room temperature, we delivered voltage-clamp pulses in cells maintained at 34–36°C. Although still lower than avian body temperature (41°C), this was the highest temperature at which we could still preserve healthy tissue and cell viability.

**FIG. 1.** 
Ca^2+^ currents activated by voltage pulses. 

A: 2 voltage pulse protocol were used. Steps made from −81-mV holding potential to values between −66 and +24 mV in 5-mV generated currents shown in B. Steps from −61 holding potential were made in 5-mV increments up to +24 mV, producing the currents shown in D. 

C: average IV relations for currents like those in B (n = 5 cells). E: average IV relations for currents like those in D (n = 5 cells). Blue traces highlight for clarity those currents with progressively declining driving force.

**FIG. 2.** Properties of low voltage–activated (LVA) Ca^2+^ currents. 

A: traces obtained by digital subtraction of a subset of currents shown in Fig. 1, B and D (with and without hyperpolarizing prepulse). B: average IV for peak currents obtained by subtraction. Currents from steps positive to −36 mV were not included because of the presence of large high voltage–activated (HVA) currents. 

C: a voltage protocol for assessing voltage dependence of steady-state inactivation of LVA current yielded a V_1/2 of −65 mV. n = 6 cells. D: exponential time constant of inactivation of subtracted records as in A, plotted as a function of voltage pulse. n = 4 cells.
maintain stable recordings. Under these conditions, Ca$^{2+}$ currents activated and inactivated much more rapidly (Fig. 3, B and C). In particular, the 10–90% rise time of currents for steps to −51 mV decreased from 8.3 ± 2.3 ms at room temperature to 2.0 ± 0.7 ms at 34–36°C (Fig. 3B). When combined with the even more rapid rise time of the high-voltage-activated channels (data not shown), these data suggest that fast electrical signals could in principle activate both kinds of Ca$^{2+}$ channel in NL neurons at physiological temperature.

Consistent with the differential channel blocker sensitivity of T-type channels compared with other, higher threshold channels (Fox et al. 1987), LVA currents were more sensitive to Ni$^{2+}$ and less sensitive to Cd$^{2+}$ than HVA. NL neurons were voltage clamped, and a step series was applied which first reprimed channels at −100 mV, jumped to −45 mV to activate and inactivate LVA, returned to −60 mV, and then jumped to 0 mV to activate HVA (Fig. 4A). This was repeated every 30 s as 100 μM Ni$^{2+}$ or Cd$^{2+}$ were washed in and out of the bath.

**FIG. 3.** Ca$^{2+}$ currents at near-physiological temperature. A: voltage pulse protocols similar to those used in Fig. 1. B: 10–90% rise time of currents obtained by subtracting traces with $V_{	ext{HOLD}}$ of −81 mV from those with $V_{	ext{HOLD}}$ of −81 mV, at either room temperature or 34–36°C, n = 5 and 6, respectively, P < 0.01. C: example records for steps from −81 mV. D: IV relation for data in C.
(Fig. 4B). In Ni\(^{2+}\), percent remaining current compared with control was 38.5 ± 3.6% for LVA and 86.6 ± 4.5% for HVA (Fig. 4C; n = 3 cells, P < 0.0004). In Cd\(^{2+}\), the remaining current was 56.0 ± 4.4 for LVA and 6.2 ± 0.9 for HVA (n = 4, P < 0.0001). LVA was inhibited significantly more by Ni\(^{2+}\) than by Cd\(^{2+}\) (P < 0.034), and HVA was inhibited significantly more by Cd\(^{2+}\) than by Nd\(^{2+}\) (P < 0.0001).

\(\text{Ca}^{2+}\) Imaging

Imaging experiments were conducted with several goals in mind. First, monitoring \(\text{Ca}^{2+}\) signals would allow us to determine to what extent \(\text{Ca}^{2+}\) channels are activated by action potentials or synaptic activity. Second, comparing signals detected in different regions of the cell would permit conclusions about the subcellular distribution of \(\text{Ca}^{2+}\) channels. Finally, by varying the resting membrane potential before triggering spikes, the relative contribution of HVA and LVA currents could be determined. Initial efforts used conventional fluorescence microscopy in which neurons were loaded with Fluo-4 or Fluo-5F through the patch pipette. This approach had the advantage of imaging entire cells in 15–50 ms and showed (Supplementary Fig. S1) that in response to single action potentials elicited in the cell body, large \(\text{Ca}^{2+}\) transients were generated simultaneously (within a single scan) throughout the dendritic arbor. A disadvantage was that, given how short NL dendrites are, it was impossible to capture an image containing a substantial portion of dendrite without the brightness of the cell body dominating the camera image.

This limitation was removed in subsequent experiments using two-photon microscopy. NL neurons were loaded with Alexa-594 and the \(\text{Ca}^{2+}\) indicator Fluo-5F through the patch pipette. After loading for 15–20 min, a low-power image of the cell was taken to assess regions to monitor at higher magnification. Line scans at regions of interest were then made for dendritic branches either near the cell body or at the outermost region of the dendritic tree. To facilitate comparisons of the signals, care was taken to avoid the larger proximal dendrites; nevertheless, small differences were evident in the diameter of proximal and distal branches. \(\text{Ca}^{2+}\) signals were monitored while evoking an action potential either by current injection through the recording electrode (1 nA, 1 ms) or by delivering a stimulus to incoming presynaptic fibers just outside the dorsal dendritic field, thereby triggering an orthodromic spike. These signals were compared for spikes triggered from either −60 or −80 mV.

Figure 5, A–C, shows examples of cells from the lateral to medial regions of NL, encompassing low to high best frequency. They show the systematic changes in cell body shape and dendrite number and length typical of chick NL (Smith 1981). Figure 5D shows voltage traces and fluorescence scans from dorsal and ventral fields from the cell in Fig. 5A. Dashed lines indicate the exact dendritic sections that were scanned. Spikes were evoked by triggering suprathreshold EPSPs, whereas the membrane potential before the spike was maintained at either −60 or −80 mV by current injection. The panel shows that large \(\text{Ca}^{2+}\) signals were apparent in both dendritic compartments, particularly when spikes were elicited from −80 mV. Figure 5E shows data from this same cell in which spikes were evoked first by current injection and then by synaptic stimulation. Large signals were readily apparent in both dorsal and ventral dendrites and for both methods of spike triggering. Figure 5G compares spikes and \(\text{Ca}^{2+}\) signals at a faster time base, showing that \(\text{Ca}^{2+}\) levels rose in all compartments during the fast phase of individual spikes.

Figure 6A shows the averaged data for the magnitude of the \(\text{Ca}^{2+}\) signal for distal and proximal locations in dorsal and ventral dendrites, at −80 and −60 mV. Locations refer to approximate distance along the dendrite, with proximal being 15–30% and distal being >85% from the soma. Signals were observed for spikes evoked by somatic current injection (Fig. 6A, blue) or for spikes arising from suprathreshold EPSPs following dorsal synaptic stimulation (Fig. 6A, red). These did not differ significantly in magnitude (e.g., Fig. 5E) and are therefore combined as the black symbols in Fig. 6A. Several conclusions can be drawn from these data. First, \(\text{Ca}^{2+}\) signals are clearly higher in more distal dendrites. Second, synaptically triggered spikes seem to trigger a global \(\text{Ca}^{2+}\) rise indistinguishable in pattern from that produced by current injection. Indeed for three cells in which both methods of spike triggering were assessed for the same section of dendrite, no significant difference was observed in the \(\text{Ca}^{2+}\) signals. Taken together, these points are consistent with a broad distribution of voltage-gated \(\text{Ca}^{2+}\) channels activated by spikes and that such spikes quickly reach all regions of the cell surface. A third point is that hyperpolarization of the neurons led to a consistent doubling of the spike-evoked \(\text{Ca}^{2+}\) signal in all dendritic compartments (Fig. 6B), indicating that the ratio of HVA and LVA channel density is constant.

A separate set of recordings was made to determine whether release from stores contributed to the dendritic \(\text{Ca}^{2+}\) transients. Slices were bathed in cyclopiazonic acid (CPA, 50 μM (Garaschuk et al. 1997) ±15 min before and during imaging experiments to eliminate store release. Responses to somatically triggered action potentials were measured at distal sites on apical dendrites. At −80 or −60 mV, the average transients were not significantly different from control cells (−80 mV: CPA, 11.35 ± 0.61, n = 5 cells/24 sites; control, 14.46 ± 1.70, n = 9 cells/18 sites, P = 0.0621; −60 mV: CPA, 8.23 ± 0.56, n = 5 cells/23 sites, control, 6.75 ± 0.72 n = 4 cells/7 sites, P = 0.19).

\(\text{Ca}^{2+}\) signals, expressed here as a ratio, are sensitive to a variety of factors, including channel density, dendritic surface area and volume, and binding properties of the dye. Segments from distal dendrites tended on average to have a smaller diameter than proximal dendrites (Fig. 6C). Accordingly, the larger \(\text{Ca}^{2+}\) transients in distal structures might be explained by their smaller volume. In some cases, however, greater than twofold differences in G/R signal was observed with little difference in process diameter. Thus we conclude that \(\text{Ca}^{2+}\) transients were greatest in the most distal processes and that \(\text{Ca}^{2+}\) channel density is either constant or increases with distance from the soma.

Subthreshold EPSPs were far less likely to generate a \(\text{Ca}^{2+}\) signal in the scanned region. EPSP amplitude was adjusted so that it would remain just subthreshold to a train of five stimuli at 100 Hz. With a single EPSP, only one scan site of one cell gave a detectable signal, out of six cells and 15 total scan sites. This cell is shown in Fig. 7A, which shows a substantial response to a single stimulus (Fig. 7C, arrow), as well as to a train. The response to a single somatic spike is also shown for

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1 The online version of this article contains supplemental data.
comparison (Fig. 7B). When trains were used, above-background dorsal dendrite signals were seen in three of six cells (15 of 25 scan sites). In two of these cells, no response was seen in ventral dendrites scans (7 sites; Fig. 7C) and in one cell, the ventral dendrites signal was 39% of the dorsal (Fig. 5F).

We suspect that this latter case may occur in small medial NL neurons having short dendritic arbors. Taken together, these data indicate that Ca\(^{2+}\) signals arising from subthreshold stimuli are extremely localized; given that subthreshold EPSPs must reflect activation of few synapses, identification of these synapses using imaging of small regions would have happened only by chance.

**DISCUSSION**

**Sources of Ca\(^{2+}\) signaling in NL**

Developing chick NL neurons express both HVA and LVA-type Ca\(^{2+}\) channels in their dendrites. These currents were larger than those recorded from the avian cochlear nucleus magnocellularis (Koyano et al. 1996; Sivaramakrishnan and Laurent 1995), mammalian ventral cochlear nucleus (octopus cells; Bal and Oertel 2007), or neurons enzymatically isolated from the mammalian dorsal cochlear nucleus (Molitor and Manis 1999). Both current types could be activated rapidly by spikes. Given that these signals arose within milliseconds of the stimulus (and thus are not likely to arise from Ca\(^{2+}\) diffusion from distant sources) and were larger in more distal dendrites than proximal ones, it is likely that HVA and LVA channels are distributed even on the most distal processes of NL.

Because NL dendrites are relatively small, it is difficult to determine directly the amplitude of the voltage transient that activated Ca\(^{2+}\) channels. Recent studies indicate that spikes in NL of hatchling chicks are initiated in the axon initial segment (Kuba et al. 2006). Apparently, in hatchling chicks, NL somata...
lacked Na\(^+\) channels and somatically recorded spikes were small, suggesting the spike only passively propagates back into the cell body and is sharply attenuated in the processes. By contrast, our data suggest, but do not prove, that, in embryonic NL, the action potential is back-propagated into dendrites. This conclusion is based on the observations that distal dendrite Ca\(^{2+}\) signals were large, that the relative contributions of HVA and LVA to those signals were independent of location, and

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

**FIG. 6.** Summary of 2-photon imaging. A: Ca\(^{2+}\) signals (\(\Delta G/R\)) for distal and proximal dendrite location in dorsal and ventral fields, as indicated. Filled (\(-80\) mV) and open (\(-60\) mV) black symbols summarize all recordings. From left to right these represent (# cells/total # scan sites) 9/18, 4/7, 3/5, 9/19. Distal dendrite signals were significantly larger than proximal signals for all but the ventral processes at \(-60\) mV (\(P < 0.01\), Student’s t-test). Red symbols are data for synaptically driven spikes. From left to right these represent (# cells/total # scan sites) 3/4, 2/3, 3/3, 3/3. Blue symbols are data for somatically triggered spikes (\(N\) as above: 7/14, 2/4, 1/2, 7/16). Dashed line shows mean for twice the SD of baseline noise (3.43 \(\pm\) 0.14, \(n\) = 75 scans). B: ratio of peak signals for recordings made at \(-80\) and \(-60\) mV as a function of dendrite location. Per cell ratio for A data, circles. C: width of scanned processes, expressed as full width half-maximum. (\(N\) from left to right: 23, 10, 5, 18 processes). Significance (*) was \(P = 0.003\) and 0.0009 for dorsal and ventral measurement sites, respectively.

**FIG. 7.** Localized Ca\(^{2+}\) signals generated by subthreshold EPSPs. A: recorded/imaged neuron showing location of recording (by cell body) and stimulus electrodes and location of scan sites (dash lines). B: spikes evoked by current injection generated similar Ca\(^{2+}\) signals in both dorsal and ventral fields. Gray traces are with resting potential = \(-60\) mV. C: train of subthreshold EPSPs (5 at 100 Hz) evoked Ca\(^{2+}\) signal in the dorsal, but not ventral, dendritic field. Arrow points to amplitude of Ca\(^{2+}\) signal from 1st EPSP.
that dorsal and ventral dendritic \( \text{Ca}^{2+} \) signals were similar. If spikes attenuated markedly along the dendrite, it might be expected that HVA contributions would also diminish along the dendrite. A secondary question concerning the source of these signals is the role of dendritic \( \text{Ca}^{2+} \) stores (Llano et al. 1994; Sandler and Barbara 1999). NL expresses high levels of both smooth endoplasmic reticulum \( \text{Ca}^{2+} \) ATPase 2 (SERCA2) (Campbell et al. 1993) and of smooth ER, which extends from the cell body into distal dendrites (Deitch and Rubel 1989). However, CPA did not significantly alter the amplitude of dendritic \( \text{Ca}^{2+} \) signals. Moreover, the very large amplitude of \( \text{Ca}^{2+} \) currents and the voltage dependence of the amplitude of \( \text{Ca}^{2+} \) signal argue for a prominent role for channels as a source of \( \text{Ca}^{2+} \).

The amplitude of the \( \text{Ca}^{2+} \) signals was strongly voltage dependent, reflecting the recruitment of LVA channels at more hyperpolarized potentials. Given a resting potential of NL neurons of near \(-60 \text{ mV} \) (Reyes et al. 1996), the larger \( \text{Ca}^{2+} \) signals we described would not likely occur, except following a hyperpolarizing stimulus. Such a stimulus could not be from GABAergic inhibitory synapses, because these are depolarizing (Funabiki et al. 1998; Yang et al. 1999), and indeed there is as yet no known biological mechanism that brings the membrane potential more negative than rest in NL.

Subthreshold synaptic stimuli generated \( \text{Ca}^{2+} \) transients that were small and much less common than spike-generated signals, as expected if they arise from activation of highly localized regions of membrane. It is likely that they are generated in part by flux through glutamate receptors. EPSPs in NL are mediated by AMPA receptors with a relatively high \( \text{Ca}^{2+} \) permeability (Raman et al. 1994; Ravindranathan et al. 2000; Zhou et al. 1995). Although it is not clear to what extent NMDA receptors also contributed to the EPSP, these receptors are expressed by NL neurons (Tang and Carr 2007). Another possibility is the occurrence of synaptically triggered local \( \text{Ca}^{2+} \)-action potentials in dendrites that do not propagate to the cell body (Golding et al. 2002). Although the small size and rarity of these signals prevented a quantitative analysis, comparison of signals triggered by subthreshold EPSPs at \(-80 \) and \(-60 \text{ mV} \) show much less difference in \( \text{Ca}^{2+} \) flux than with spike-triggered signals (Figs. 5F and 7C), suggesting a role for relatively voltage-insensitive \( \text{Ca}^{2+} \) channels. Thus dendritic sources of \( \text{Ca}^{2+} \) differ sharply as a function of stimulus. Spikes triggered anywhere on the cell led to a \( \text{Ca}^{2+} \) rise from activation of voltage-dependent \( \text{Ca}^{2+} \) channels. Subthreshold EPSPS probably elevated \( \text{Ca}^{2+} \) in part using glutamate receptors. Therefore postsynaptic sites generating suprathreshold EPSPS generated local signals from both synaptic receptors and voltage gated \( \text{Ca}^{2+} \) channels.

**Potential roles of \( \text{Ca}^{2+} \) signaling in NL**

Synaptic activity has been proposed to mediate growth and restructuring of dendritic arbors (Cline 2001; Wong and Ghosh 2002). Deafferentation in NL results in a rapid partial withdrawal of the denervated dendrite (Benes et al. 1977; Deitch and Rubel 1984; Sorensen and Rubel 2006), and it has been proposed that synaptic activity, and possibly associated \( \text{Ca}^{2+} \) flux, drives or regulates these morphological responses (Deitch and Rubel 1989; Sorensen and Rubel 2006). Embryonic development of NL is characterized by a striking remodeling of dendrites (Smith 1981). This remodeling has two consequences. First, dorsal and ventral dendrite lengths become roughly matched in length and number, a key adaptation to efficient coincidence detection of signals from ipsi- and contralateral inputs (Agnom-Snir et al. 1998; Grau-Serrat et al. 2003). Second, the length of dendrites becomes graded according to the best frequency of sound they process. Although it is not certain whether NL neurons are electrically active during this developmental period in vivo, it has been shown that afferent synapses from nucleus magnocellularis have already formed (Hendricks et al. 2006) and that auditory nerve fibers synapsing on magnocellularis neurons are spontaneously active (Jones et al. 2001; Lippe 1994) and can drive magnocellularis neurons (Jackson et al. 1982) Given that magnocellularis inputs to NL can also drive spikes (this study and Gao and Lu 2008), it is likely that that NL cells fire spontaneously. Therefore it is plausible that either local or global \( \text{Ca}^{2+} \) signals occur in NL dendrites during development and that these may play a role in dendritic growth or stabilization.

Our results indicate that the role of \( \text{Ca}^{2+} \) transients will differ dramatically, depending on whether they are driven by subthreshold activity or spikes. If one proposes that the length of a dendrite is regulated independently of its neighbors by \( \text{Ca}^{2+} \)-dependent processes, it would be essential that EPSPs be subthreshold, because spikes in NL neurons would inevitably produce similar \( \text{Ca}^{2+} \) rises throughout the whole cell. Lohmann et al. (2002) showed that such local, but not global, \( \text{Ca}^{2+} \) transients were essential to maintain dendritic processes. These local changes were caused by release from stores, and it was suggested that a global \( \text{Ca}^{2+} \) rise caused by VGCCs might not trigger stores or could suppress the effects of store activation. It will therefore be necessary to examine local \( \text{Ca}^{2+} \) transients in NL and their origin. Alternatively, it may be that, in NL, spike-driven global rises in \( \text{Ca}^{2+} \) provide an “equalizing” signal to drive similar growth or stabilization of processes at all dendrites. This could be consistent with the similarity in dorsal and ventral dendrites but not with the observation that unilateral deafferentation produces unilateral dendritic retraction. Therefore it seems likely that synapse-dependent signals that act on specific dendrites must involve additional signals besides \( \text{Ca}^{2+} \), such as activation of metabotropic receptors or release of neurotrophic substances (Sorensen and Rubel 2006).

We do not know whether dendritic \( \text{Ca}^{2+} \) channels are maintained into adulthood; imaging in such tissue is quite challenging. However, large \( \text{Ca}^{2+} \) currents have been documented in mammalian octopus cells of juvenile mice, cells that have similar electrical properties to MSO and NL neurons (Bal and Oertel 2007; Golding et al. 1999). If adult NL neurons maintain these channels, they might provide a local boost to EPSPs, provided their kinetics were sufficiently rapid. Alternatively, they could provide an ongoing “metric” of average electrical activity in the form of calcium ions that could serve to drive homeostatic mechanisms (Bal and Oertel 2007).
REFERENCES


