Visualization of Calcium Influx Through Channels That Shape the Burst and Tonic Firing Modes of Thalamic Relay Cells

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Zhou, Qiang, Dwayne W. Godwin, Donald M. O’Malley, and Paul R. Adams. Visualization of calcium influx through channels that shape the burst and tonic firing modes of thalamic relay cells. J. Neurophysiol. 77: 2816–2825, 1997. Thalamic neurons have two firing modes: “tonic” and “burst.” During burst mode, both low-threshold (LT) and high-threshold (HT) calcium channels are activated, while in tonic mode, only the HT-type of calcium channel is activated. The calcium signals associated with each firing mode were investigated in rat thalamic slices using whole cell patch clamping and confocal calcium imaging. Action potentials were induced by direct current injection into thalamic relay cells loaded with a fluorescent calcium indicator. In both tonic and burst firing modes, large calcium signals were recorded throughout the soma and proximal dendrites. To map the distribution of the channels mediating these calcium fluxes, LT and HT currents were independently activated using specific voltage-clamp protocols. We focused on the proximal region of the cell (up to 50 μm from the soma) because it appeared to be well clamped. For a voltage pulse of a given size, the largest calcium signals were observed in the proximal dendrites with smaller signals occurring in the soma and nucleus. This was true for both LT and HT signals. Rapid imaging, using one-dimensional linescans, was used to more precisely localize the calcium influx. For both LT and HT channels, calcium influx occurred simultaneously throughout all imaged regions including the soma and proximal dendrites. The presence of sizable calcium signals in the dendrites, soma, and nucleus during both firing modes, and the presence of LT calcium channels in the proximal dendrite where sensory afferents synapse, have implications for both the electrical functioning of relay cells and the transmission of sensory information to cortex.

INTRODUCTION

Calcium currents in thalamic relay cells influence the flow of sensory information through the thalamus. Thalamic transmission is gated by the two firing modes of thalamic relay cells: burst and tonic (Steriade and Llinas 1988). The burst mode is directly dependent on the availability of a specific type of calcium channel, the low-threshold (LT) channel. In this mode, the LT calcium current (or T current) is activated via a fairly modest depolarization, resulting in an LT spike (Crunelli et al. 1989; Deschenes et al. 1984; Hernandez-Cruz and Pape 1989; Jahnsen and Llinas 1984a,b; reviewed in Huguenard 1996). This LT spike, a triangular depolarization underlying a rapid burst of two to seven sodium action potentials, is followed by a significant refractory period, due in part to inactivation of LT channels. The flow of sensory information through the thalamus is therefore highly nonlinear during burst mode. Relay cells exhibit this burst mode only when they are hyperpolarized to about −65 mV or more. At more depolarized membrane potentials, the LT channels are inactivated and only the high-threshold (HT) calcium channels are available for calcium entry. Thalamic relay cells are then in a tonic firing mode: sustained input causes sustained (tonic) firing of action potentials. This tonic firing allows the duration and amplitude of depolarizing inputs to be encoded in a more linear fashion than is possible during burst mode (Deschenes et al. 1984; Guido et al. 1992, 1995; Jahnsen and Llinas 1984a; Lu et al. 1992; McCormick and Feeser 1990).

HT and LT channels will have a range of influences on relay cell functioning. First, the electrical currents they generate contribute to the overall excitability of thalamic neurons. A detailed mapping of their distribution would therefore help to define the “spatial excitability” of the cell’s dendritic tree (Llinas 1988; Midtgaard 1994; Yuste et al. 1994). Second, modulation of these channels, by local electrical events or second-messenger systems, might be spatially delimited by the anatomic distribution of the channels, especially because cortical and subcortical inputs to relay cells often innervate distinct regions of a relay cell’s dendritic tree (Kultas-Illinsky and Illinsky 1991; Liu et al. 1995; Peschanski et al. 1984, 1985; Wilson et al. 1984). Finally, the calcium signals generated via LT and HT calcium channels may produce downstream events such as the modulation of ionic conductances (e.g., potassium and calcium channels) (Marty 1989), and the induction of calcium-dependent signaling cascades, long-term potentiation, and other intracellular/intranuclear processes (Bading et al. 1993; Komatsu and Iwakiri 1992; Miller 1988).

Anatomic studies of the distribution of HT calcium channels (including L, N, and P) on hippocampal, cerebellar, and cortical cells indicate that high-threshold channels are distributed throughout the soma and dendritic tree, with each specific type of channel usually showing a distinct labeling pattern (see, e.g., Hillman et al. 1991; Mills et al. 1994; Westenbroek et al. 1992, 1995). In the case of LT channels, highly specific ligands or antibodies are not available, so physiological methods are required. On the basis of polarity changes observed in extracellular recordings of inferior olive neurons, Llinas and Yarom (1981) suggested that LT channels have a predominantly somatic localization while the HT channels are concentrated more on the dendrites. In contrast, studies of hippocampal pyramidal cells, using single-channel recordings and fluorescent calcium imaging, found that the LT channels were less concentrated on the soma than the HT channels (Christie et al. 1995; Magee and Johnston...
1995a; but see Markram et al. 1995). Although the methods in the early paper of Llinas and Yarom are less direct, the inferior olivary neurons might well be organized differently than hippocampal pyramidal cells.

Here we are concerned with thalamic relay cells, which are in a sense analogous to inferior olive neurons because the thalamus and the inferior olive both relay sensory information to cortical structures (cerebral and cerebellar cortex, respectively). A detailed mapping of calcium channels in thalamic relay cells has not been done, but a computational analysis of currents recorded from thalamic reticular neurons suggests a high density of LT channels in the distal dendrites (Destexhe et al. 1996). We have adopted the strategy of using voltage clamp to selectively activate LT and HT calcium channels in thalamic neurons (from the ventrobasal and lateral geniculate nuclei), while simultaneously monitoring calcium influx using confocal calcium imaging. Preliminary versions of this work have been reported in abstract form (Zhou et al. 1995, 1996).

METHODS

Slice preparation

Postnatal rats between 10 and 18 days old were briefly anesthetized with ketamine (33 mg/kg) before decapitation. The thalamus was separated from the rest of the brain and sliced with a vibroslicer (Campden Instruments) at 200- to 300-μm intervals in ice-cold cutting solution containing (in mM) 125 NaCl, 1 MgCl₂, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, and 25 glucose. The solution was bubbled continuously with 95% O₂-5% CO₂. The slices were held in the same solution at 33°C until use. Within 1–4 h after cutting the slices, recordings were made from the lateral geniculate nucleus (LGN) or the ventrobasal (VB) nucleus. Although the exact boundary of the VB nucleus is not always obvious, many of the recordings were made near the center of the VB nucleus. The optical/patch-clamp recordings were done at room temperature. This facilitates the localization of calcium channels because of the slowing of channel kinetics, which improves voltage control or “space clamp,” a key concern in these studies. The slowing of the channel kinetics and calcium diffusion may also aid in the optical localization of the channels. The voltage dependence of the activation and inactivation of the LT channels, however, is reported to be the same at room and physiological temperatures (Crunelli et al. 1989).

Electrophysiological recordings

Thalamic neurons were recorded in whole cell mode under visual guidance, after Edwards et al. (1989). Slices were placed in a perfusion chamber at room temperature (22–24°C) on an upright Zeiss Standard 16 microscope and imaged with a Zeiss ×40 water immersion objective. The cells were visualized with a Dage-MTI CCD-72 video camera to facilitate patching. Recording electrodes were pulled from thin-wall glass (World Precision Instruments) and had input resistances between 4 and 8 MΩ. In current-clamp experiments, tonic firing was evoked by holding cells at −55 to −50 mV and injecting a depolarizing current pulse, causing repetitive firing of the cell. Burst firing was evoked by injection of a depolarizing current pulse from a more hyperpolarized membrane potential (−70 mV or more negative). The external solution for current-clamp experiments contained 4 mM CaCl₂, but was otherwise the same as the slicing solution. The internal solution contained (in mM) 128 potassium glutonate, 10 NaCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-Na, 2 MgCl₂, and 2 Na₃ATP. In voltage-clamp experiments, cells were held at −50 mV, and depolarizing voltage steps were used to activate HT current. To activate LT current, cells were first hyperpolarized to −100 or −110 mV for 500 ms and then depolarized. Discontinuous voltage clamp (using an Axoclamp 2A amplifier and Pclamp software) was used to record calcium currents; headstage waveforms were monitored continuously to ensure proper settling.

The external solution contained (in mM) 100 NaCl, 1 KCl, 2 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 2 CaCl₂, 5 4-aminoypyridine, 20 tetraethyl ammonium-chloride, 25 glucose, and 0.5 μM tetrodotoxin (TTX). The internal solution contained (in mM) 115 CsCl, 6 MgCl₂, 10 HEPES-Cs, 4 Na₃ATP, 0.4 Na₂GTP, 15 phosphocreatine, and 50 U/ml creatine phosphatase.

Confocal imaging

A BioRad MRC 600 confocal imaging system was used to image calcium responses in patch-clamped neurons loaded with calcium green dextran, either 100 or 300 μM (in earlier experiments fluo 3 was used with similar results; indicators are from Molecular Probes). Cells were allowed to load with indicator for ≥10 min until the region of interest was bright enough to image with a low level of illumination (the 488-nm line of a 15-mW argon-krypton laser was usually attenuated by 90%). Two-dimensional images were acquired at 470-ms intervals. Faster one-dimensional images, “line scans,” were made by repeatedly scanning a single line of interest at 2-ms intervals. Acquisition of electrophysiological and imaging data were synchronized with the use of a pulse from an interval generator (WPI). The size of the calcium responses in different regions of cells loaded with these indicators is roughly proportional to the relative fluorescence change (ΔF/ΔF) (O’Malley 1994). Although information about absolute calcium levels is desirable, our studies concern the relative size of LT and HT calcium signals within individual cells, so absolute calcium levels are not essential. To indicate the magnitude of these responses, if we assume a typical resting free calcium level of 100 nM, then a 10% increase in calcium would equate to about a 20-nM calcium increase, whereas a 100% ΔF/ΔF would correspond to a 490-nM increase. These calculations were based on our intracellular determination of the Kₘ (250 nM) and dynamic range (8.7-fold) of calcium green dextran (O’Malley et al. 1995).

Analysis

In voltage-clamp experiments, data from cells or trials with significant deviations from the command voltage were discarded. Also, to minimize the contamination of HT signals by HT currents, only those trials in which the LT current was clearly transient were included. Total charge entry was calculated by integrating the current trace over the period when the calcium current was activated. Fluorescence responses were quantified with the use of a measuring box over the region of interest in frame scan experiments, or by quantifying the fluorescence line by line in line-scan experiments. After subtraction of background, the fluorescence values for each region were plotted by normalizing to the basal fluorescence value in each region at the outset of each trial. When imaging soma and dendrites simultaneously, the dendritic signals were in some instances close to background fluorescence levels (black level + autofluorescence). Under this condition, any errors in background subtraction would produce significant errors in ΔF/ΔF, so care was taken to make this correction as accurately as possible. A Wilcoxon signed-rank test (1-tailed) was used for statistical comparisons. In plotting some of the line scans, a running average of three consecutive data points was used to smooth line to line noise.

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RESULTS

Neuronal somata in the VB nucleus and the LGN were visualized with the use of contrast-enhanced video microscopy, which facilitated patch clamping and whole cell recording (Fig. 1A). Recordings were made from larger neurons (15–30 μm diam) in the slice, because they are more likely to be relay cells than interneurons. Also, filling cells with fluorescent dye revealed at least three primary dendrites, which is a further indicator of relay neurons (Leresche 1992). In addition, many of the recordings were made specifically from the ventrobasal nucleus of the thalamus, which has virtually no interneurons (Harris 1986). Hence the majority of the recordings are from thalamic relay cells. In recordings made using current clamp, burst and tonic modes were selectively activated by holding the cells \( n = 15 \) at different resting membrane potentials before depolarization. HT calcium currents were activated in tonic mode by holding cells between -50 and -55 mV and injecting positive current. This produced a pattern of action potentials (Fig. 1B, top trace) similar to that occurring during tonic firing in vivo. In contrast, hyperpolarizing the same cell to -70 mV, and then injecting positive current, induced a low-threshold calcium spike (Fig. 1B, bottom trace), a response that depends on the activity of LT calcium channels, but also includes a component from HT channels that are activated during the burst of sodium action potentials (Jahnsen and Llinas 1984a,b).

To simultaneously record calcium signals, calcium green dextran had been included in the patch-clamp pipette. Also, 4 mM CaCl\(_2\) was used in the external solution during current-clamp experiments to improve signal detectability. Calcium signals appeared in the soma and proximal dendrite during both tonic- and burstlike firing modes. The stimulus was adjusted so that the same number of action potentials occurred in both firing modes. A larger calcium increase was typically observed in the burst mode for any given number of action potentials (1–3 action potentials were evoked in different experiments). In bursts containing three action potentials (within the range of typical LT spikes), the burst...
signals were 10–25% larger than the tonic signals (Fig. 1C, same cell as Fig. 1B). This was expected because both LT and HT channels are activated in burst mode, whereas only the HT calcium channels are activated in tonic mode. This also suggests that in the soma, during an LT spike, the HT channels carry the bulk of the calcium, with the LT channels carrying a smaller fraction. In both firing modes, the somatic calcium signals rose sharply at the onset of stimulation and recovered gradually over a few tens of seconds. This recovery time scale is similar to that seen in neuronal somata in slice preparations from hippocampus, cerebellum and cortex, and also in CNS neurons in vivo (Regehr and Tank 1992; Lev-Ram et al. 1992; Yuste et al. 1994; Fethco and O’Malley 1995).

These rapid calcium increases, observed during both tonic and burst firing modes, suggest that both LT and HT currents were present throughout the soma and proximal dendrites. However, current-clamp recordings do not completely dissociate the two calcium currents, so we used voltage-clamp protocols to selectively activate LT and HT channels in relay cells (n = 44). The distribution of HT calcium responses was mapped first. After 10–15 min of filling with calcium green dextran, there was usually a fair amount of labeling of the soma and the most proximal dendrites; more detailed labeling was obtained by 60 min (Fig. 2A). Cells were held at −50 mV and depolarized to 0 mV to activate the HT channels. Depolarizing the cells (for 100 ms in Fig. 2B) induced a substantial calcium increase in the nucleus, cytosol, and an immediately adjacent dendrite, with the dendritic response being the largest (Fig. 2B2). These changes are representative of the larger population (n = 28 cells, P < 0.01).

The presence of calcium increases throughout the soma and proximal dendrites suggests that HT channels are widely distributed. However, diffusion of calcium from a distance could also account for the response, because the individual images were acquired relatively slowly, at 470-ms intervals. Rapid measurement with one-dimensional line scans allowed a more direct mapping of the channels. A scan of the line in Fig. 2C showed that shortly after the onset of depolarization, calcium increased rapidly across all scanned regions of the cell. These calcium dynamics were quantified by plotting the relative fluorescence increase at different locations across the cell (Fig. 2D). The increases in fluorescence at the soma and dendrite begin within 4 ms of one another and show a similar time course, consistent with calcium influx occurring simultaneously throughout this region of the cell.

In other cells, line scans were made to cross dendrites at varying distances from the cell soma. The fluorescence signals again increased rapidly in all cellular regions examined, i.e., within ∼4 ms of each other, out to a distance of 40–50 μm from the cell body, and including dendrites up to the third order (Fig. 2E). Considering that in cultured neurons it takes ∼40 ms for a calcium wave to diffuse 5 μm from the plasma membrane (see, e.g., Hernandez-Cruz et al. 1990; O’Malley 1994), we can conclude for the essentially synchronous calcium increases observed here that calcium sources must be located within ∼2 μm of these imaged regions. Similar results were observed in all neurons where HT calcium currents were mapped, indicating that these channels are widespread (n = 12). Although “patchy” channel distributions were not observed, the patches observed in anatomic studies of hippocampal neurons are often separated by rather small distances and would not easily be resolved by our methods. The relative fluorescence increases were largest in the dendrites including the most distal regions imaged, smaller in the soma and smallest in the nucleus (n = 7, P < 0.01). To rule out a contribution of N-methyl-D-aspartate (NMDA) receptors to these responses, these experiments were repeated in the presence of the NMDA receptor blocker 2-amino 5-phosphonopentanoic acid (AP5). AP5 did not cause any obvious decrease in the responses, indicating that any contribution from NMDA receptors was minimal (n = 3, data not shown).

To selectively activate LT calcium channels, cells were hyperpolarized to −100 or −110 mV for 500 ms and then depolarized to −40 or −50 mV. Current-voltage (I-V) curves showed that little (<5%) of the HT current was activated by this protocol, in agreement with other studies (Coulter et al. 1989; Hernandez-Cruz and Pape 1989). In conjunction with our I-V curves, we also measured the fluorescence response as a function of voltage; the integrated current and the calcium responses are expected to roughly coincide if the voltage control is adequate. We observed a close correspondence between the charge-voltage (Q-V) and the fluorescence-voltage (F-V) curves, as shown in Fig. 3A where low- and high-threshold currents were sequentially evoked by a series of voltage steps from a holding membrane potential of −110 mV. In the Q-V curve, the initial plateau represents the LT current while the large peak at +10 mV represents HT current. This separation of currents is also seen in the F-V curve acquired from the soma: the simultaneously recorded fluorescence responses parallel the Q-V curve, indicating good voltage clamp. Similar results were obtained in cell somata and in proximal dendrites up to 50 μm from the soma (n = 10).

An example of the somatic LT calcium signal is shown in Fig. 3B. The cytosolic signal peaks in image 4 (B1, 1st frame in 2nd row), which was collected immediately after the depolarization. The fluorescence response is plotted in Fig. 3B2. Significant responses were seen in all cells where LT responses were mapped (n = 24, P < 0.01). To compare the distribution of LT and HT channels, appropriate voltage protocols were applied sequentially within individual cells. LT and HT responses in the soma and proximal dendrites were compared with the use of line scans (Fig. 3C). Currents and calcium signals are shown for both LT (top panel) and HT currents (bottom panel) for the soma and a dendritic region 12 μm from the soma. Calcium influx through LT and HT channels occurred simultaneously in both regions. Both LT and HT signals increased from the soma to the dendrites, with the LT fluorescence signal tending to increase relatively more. Simultaneous calcium increases were evident for both channel types throughout all imaged regions, including regions up to 50 μm from the soma including both VB and LGN neurons (n = 10).

If the distribution of the calcium channels (LT or HT) were uniform, we would expect larger dendritic responses, because dendrites have a larger surface to volume (S/V) ratio than the soma. An exact quantitation of channel density from these data is, however, problematic, because of uncertainties in absolute calcium levels. A rough estimate of the
surface to volume ratios of soma and dendrites of the cell studied in Fig. 3C yielded a dendritic S/V ratio that was 3.3-fold times the somatic S/V ratio. If we assume a resting free calcium level of 50 nM, then the dendritic calcium increase (see METHODS) is 3.4-fold greater than the somatic increase for the LT channels and 2.9-fold greater for the HT channels—values in the range expected for a uniform distribution. However, if a resting calcium level of 100 nM is assumed, then the LT calcium signal increases by 5.3-fold from soma to dendrite and the HT signal by 7.7-fold,
and so the channel density would be increasing from soma to dendrites. Indeed, at the higher assumed calcium level, although the LT signal showed the largest relative increase, the HT signal, because of its higher absolute increase (and the nonlinear relationship between calcium and fluorescence) actually increased more than the LT calcium response. These limits to interpretation are not simply a matter of our imaging methods or choice of calcium indicator, because even in the most intensively studied case of muscle calcium, estimates of resting free calcium range between 30 and 300 nM (Baylor et al. 1994; Westerblad and Allen 1994). Also, a detailed calibration curve demonstrating the in vivo $K_D$ and dynamic range of a ratiometric indicator inside a nerve cell is yet to be published.

**DISCUSSION**

Previously there was little information about the distribution of LT and HT calcium channels across thalamic relay cells, although a computational model of the distribution of calcium currents indicated that in thalamic reticular neurons, the major portion of the LT channels are located distally (Destexhe et al. 1996). Here we have shown that both LT and HT calcium channels are widely distributed across the soma and proximal dendrites of relay neurons, at least as far as 50 $\mu$m from the soma. Moreover, the use of line scans confirmed that both channel types were present in all imaged regions, ruling out diffusion of the calcium from remote sites. Similar channel distributions were observed in all thalamic relay neurons studied, including VB and LGN neurons, which constitute the major routes by which somatosensory and visual information, respectively, enter mammalian cortex.

One approach to localizing HT and LT channels is to pharmacologically eliminate one channel type and then study the other type in isolation. However, highly specific blockers of the LT channels are not yet available. Concentrations of nickel, for example, that block LT channels also affect HT channels (Christie et al. 1995; Huguenard and Prince 1992). Conversely, HT currents can be partially blocked by a mixture of compounds, but complete blockade also inhibits LT currents (Guyon and Leresche 1995; Huguenard and Prince 1992). Our approach was to selectively activate the LT or HT calcium currents using standard voltage-clamp protocols. The key limitation to this approach is the ability of the clamp to control dendritic voltages at increasing distances from the soma. However, thalamic neurons are thought to be electrically “compact” (Bloomfield et al. 1987; Crunelli et al. 1987), and the typical electrotonic length constant of neurons is well in excess of the distances being studied here. For example, Stuart and Hausser (1994) report a steady-state voltage attenuation of 20% at 150 $\mu$m from the soma in cerebellar Purkinje cells. Because we have focused our efforts on the first 50 $\mu$m of the cell, we expect the deviation from the command voltage to be small. This is supported by the similarity of the $F$-$V$ and $Q$-$V$ curves in the soma and proximal dendrites (Fig. 3A and unpublished results).

Proximal calcium currents are significant for several reasons. Channels in this region, by virtue of their proximity to the axon hillock, are well positioned to influence the cell’s firing. Furthermore, the proximal regions of relay cells receive the main sensory afferents to thalamus. This includes the retinal afferents to LGN, somatosensory afferents to VB, and other major afferents such as the deep cerebellar afferents to ventrolateral nuclei (Wilson et al. 1984; Peschanski et al. 1984, 1985; Kultas-Ilinsky and Ilinsky 1991; Liu et al. 1995). Parabrachial brain stem afferents also synapse proximally onto thalamic relay cells (Wilson et al. 1984), and their stimulation switches relay cells from burst to tonic mode (Hu et al. 1989; McCormick and Prince 1987; Lu et al. 1993). Because HT calcium channels are widely modulated by neurotransmitters and second-messenger systems (see, e.g., Bean 1989; Dolphin 1990; Dunlap and Fischbach 1981; Hille 1994), as are the LT channels to a more limited extent (Berger and Takahashi 1990; Toselli and Lux 1989; reviewed in Huguenard 1996), their colocalization with specific inputs allows for potentially specific modulation, especially given that local increases in second-messenger signals have been demonstrated in several systems (Bacskaï et al. 1993; Hahn et al. 1992). One further possibility is that the activated calcium channels themselves contribute to the modulation of relay cell activity, through calcium-dependent signaling pathways (Baimbridge et al. 1992; Hanson and Schulman 1992; Marty 1989; Miller 1988).

Of particular interest is the colocalization of LT channels with the retinal afferents to LGN cells. Because LT channels help bring hyperpolarized thalamic relay cells to threshold, this arrangement could serve as an amplifier by boosting retinal excitatory postsynaptic potentials (EPSPs) (Lu et al. 1992). Activation of LT channels by EPSPs has been demonstrated in other systems including hippocampal slices (Magee and Johnston 1995b) and neocortical neu-
Comparison of calcium influx through low-threshold (LT) and HT calcium channels. 

A: fluorescence-voltage (F-V) and charge-voltage (Q-V) curves from a VB neuron. With the use of voltage clamp, a series of 100-ms-long voltage steps from −110 mV to increasingly higher voltages was given, while simultaneously measuring current and somatic fluorescence. Charge influx (measured by integrating current over the duration of the voltage step) and fluorescence increase are plotted for each step. Charge and fluorescence are normalized to −100 (peak values: fluorescence: 152% increase; charge: 305 pC). Insets: calcium currents recorded at −34 mV (LT, transient) and +8 mV (HT, sustained). B: somatic fluorescence increase on activation of LT calcium current in a VB neuron. B1: just before frame 4 (1st image in 2nd row) the cell was depolarized from −110 to −35 mV for 100 ms. B2: time course of fluorescence signal from measuring box shown in B1 (470 ms per data point). C: comparison of LT and HT calcium signals in a VB neuron. A line crossing the soma and proximal dendrite (12 μm from the soma) was scanned. C1: activation of LT channels (−110 to −40 mV, 100-ms pulse) causes synchronous calcium increases in the soma and branch. C2: activation of HT (−50 to 0 mV, 30-ms pulse) produces similar results. Insets: current recorded for each voltage step; current records were filtered at 1 kHz.

rons (Deisz et al. 1991; but see Stuart and Sakmann 1995). That retinal inputs might trigger LT spikes was suggested by the production of LT spikes on stimulation of the optic tract in thalamic slices (Scharfman et al. 1990; Turner et al. 1994). Moreover, in awake behaving cats, the initial appearance of a visual stimulus in a relay cell’s receptive field can be accompanied by an LT spike (Guido and Weyand 1995). Thus LT spikes, in addition to their well-established role in generating oscillatory activity such as occurs in sleep (Bal et al. 1995; Steriade and Llinas 1988),
may also help to transmit sensory information to cortex in a nonlinear mode. Although such nonlinear transmission could enhance the detectability of visual signals (Godwin et al. 1996; Guido et al. 1992, 1995; Lu et al. 1992), behavioral consequences of stimulus-evoked bursts remain to be demonstrated (Sherman and Guillery 1996). The substantial numbers of LT channels that are present on the soma and proximal dendrites could underlie such a function. Although colocalization of LT channels and retinal inputs is not a necessary condition for boosting retinal signals, it may be the most effective arrangement.

One caveat to such a proposed role for the proximal LT channels is that our observations have been made on young [postnatal day 10 (P10) to P18] rats, where the visual system is in an early stage of development. The use of young rats was necessitated by the need for an optically clear preparation. The calcium channels we observe at this stage are presumably assisting in the dynamic organization of the rat visual system. For example, at P18, neurons in rat visual cortex show immature receptive fields that are quite large and binocular (Fagiolini et al. 1994). Other studies have reported that the size of LT currents and bursts increases markedly in early postnatal life in both cats (Pirchio et al. 1990) and ferrets (Ramoa and McCormick 1994), and we see a similar progression toward adultlike LT spikes over the P10–P18 interval (unpublished observations). Currently, there is no direct evidence that adult thalamic neurons have an LT channel distribution similar to that observed here. However, in thalamic slices from adult cat (Scharfman et al. 1990) and adult rat (Turner et al. 1994) and in intact adult cats (Guido and Weyand 1995; Lu et al. 1992), optic nerve activity produces LT spikes. Those results are consistent with the idea that LT channels are located on the soma and/or proximal dendrites of adult relay neurons.

In both tonic and burst mode, substantial calcium increases occur throughout the soma, nucleus, and proximal dendrite. In tonic firing mode, large calcium signals were recorded during sustained (200 ms) depolarizations, which produced sustained firing, as can occur in response to sensory stimulation (Lu et al. 1992). Calcium signals this large were never produced in burst mode, even with a 200-ms stimulation. Rhythmic activity in vivo, however, might produce quite sustained periods of bursting, possibly producing greater signals than we observed in the slice. Indeed, we observed summation of calcium signals during short trains of bursts at frequencies in the lower range of spindle wave frequencies (Zhou et al. 1996). Thus the nucleus of a thalamic relay cell might exhibit a sustained elevation of calcium during burst mode, e.g., in different stages of sleep, and perhaps in pathological states such as absence seizures (Bal et al. 1995). The possible cellular responses to these proximal calcium fluxes are myriad, yet poorly understood. For example, calcium stimulates gene expression (e.g., Bading et al. 1993; Enslen et al. 1994; Murphy et al. 1991), but the exact levels of calcium required for induction of specific genes is generally not known. Although a fuller understanding of the significance of these proximal calcium signals awaits further study, the present results indicate that the LT and HT channels should have significant electrical influences on the firing of thalamic relay cells and that the LT channels may contribute to the amplification of sensory inputs.

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