

Effects of Membrane Voltage on Receptive Field Properties of Lateral Geniculate Neurons in the Cat: Contributions of the Low-Threshold Ca^{2+} Conductance

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SUMMARY AND CONCLUSIONS

1. Thalamic relay cells, including those of the lateral geniculate nucleus, display a low-threshold spike (LT spike), which is a large depolarization due to an increased Ca^{2+} conductance. Typically riding the crest of each LT spike is a burst of from two to seven action potentials, which we refer to as the LT burst. The LT spike is voltage dependent, because if the cell's resting membrane potential is more depolarized than roughly -60 mV, the LT spike is inactivated, but if more hyperpolarized, the spike is deinactivated and can be activated by a depolarization, such as from an afferent excitatory postsynaptic potential (EPSP). Thalamic relay cells thus display two response modes: a relay or tonic mode, when the cell is depolarized and LT spikes are inactivated, leading to tonic firing of action potentials; and a burst mode, when the cell is hyperpolarized and tends to respond with LT spikes and their associated bursts of action potentials.

2. We were interested in the contribution of the LT spike on the transmission of visually evoked signals through geniculate relay cells to visual cortex. We recorded intracellularly from geniculate cells in an anesthetized, paralyzed, *in vivo* cat preparation to study the effects of membrane voltage, and thus the presence or absence of LT spikes, on responses to drifting sine-wave gratings. We monitored the visually evoked responses of 14 geniculate neurons (6 X, 7 Y, and 1 unclassified) at different membrane potentials at which LT spikes were inactivated or deinactivated.

3. Changing membrane voltage during visual stimulation switched the response mode of every cell between the relay and burst modes. In the burst mode, LT spikes occurred in phase with the visual stimulus and not at rhythmic intervals uncorrelated to visual stimuli. To any given stimulus cycle, the cell responded usually with an LT burst or a tonic response, and rarely was more than one LT burst evoked by a stimulus cycle. Occasionally a single cycle evoked both an LT burst and tonic response, but always the LT burst occurred first.

4. The spatial tuning characteristics of the cells did not differ dramatically as a function of membrane potential, because the tuning of the LT bursts was quite similar to that of the tonic response component. Although we did not obtain complete temporal tuning properties, we did note that hyperpolarized cells responded reliably with LT bursts at several temporal frequencies.

5. A consistent difference was seen between the LT burst and tonic response components in terms of response linearity. We measured this by computing the fundamental and second harmonic Fourier amplitudes of the responses (F1 and F2, respectively). The F1 amplitude represents the linear portion of the response, and the F2 amplitude represents a measure of response nonlinearity. We found that, for every cell, the F2-to-F1 ratio was considerably higher for the LT burst than for the tonic response component.

6. We found that the bursts associated with LT spikes had interspike intervals ≤ 4 ms. However, we noted that, during relative

depolarization, cells could respond during the relay mode with bursts also having interspike intervals ≤ 4 ms. This seemed simply to reflect increasingly high firing rates as the cell became more depolarized. We refer to this bursting during the relay firing mode as high-threshold (HT) bursts, because they were not associated with LT spikes and thus arose from the higher threshold associated with conventional action potentials.

7. Finally, we were able to develop reliable empirical criteria to distinguish the LT burst from the tonic response component, criteria that were based solely on the temporal pattern of action potentials. We found that, because of HT bursts, the brief interspike interval of the LT burst was an insufficient criterion. However, the LT burst was also characterized by a prior silent period not seen during HT bursts. This silent period was > 100 ms, except when we used visual stimuli at temporal frequencies > 8 Hz; for higher frequencies, we found that a silent period ≥ 50 ms followed by brief interspike intervals successfully identified an LT burst. This permits the use of extracellular recording to study LT spiking, thereby providing a more practical means of quantitatively studying the separate contributions of the LT burst and tonic response components to visual responsiveness.

INTRODUCTION

It is now quite clear that the lateral geniculate nucleus of mammals does not perform a simple, invariable relay of retinal information to cortex (Burke and Cole 1978; Sherman and Koch 1986, 1990; Singer 1977; Steriade and Llinás 1988). Both morphological and electrophysiological data indicate that geniculate circuitry acts to control the gain of retinogeniculate transmission (Sherman and Koch 1986, 1990). Morphologically, retinal terminals represent only ~ 10 – 20% of synaptic inputs onto relay cells, suggesting that there is more to geniculate circuitry than simply subserving the relay of this relatively small input. The nonretinal input, which represents the vast majority, derives from descending corticogeniculate axons, local inhibitory circuits, and ascending axons from the brainstem reticular formation. Electrophysiologically, thalamic relay cells, including those of the lateral geniculate nucleus, exhibit a number of nonlinear, voltage-dependent membrane properties or ionic conductances. These conductances can greatly alter the nature of retinogeniculate transmission.

One of the most dramatic of these conductances is the voltage-dependent, low-threshold (LT) Ca^{2+} spike (Deschênes et al. 1984; Jahnsen and Llinás 1984a,b). This is an increase in conductance to Ca^{2+} , which leads to a

triangular, spikelike depolarization of the cell. It is called "low threshold" because its initiation threshold is lower than that of a conventional action potential. However, when the membrane is more depolarized than roughly -60 mV, this conductance becomes inactivated; the membrane must be hyperpolarized below this level for ≥ 50 – 100 ms to deinactivate the conductance so that the next sufficiently large depolarization will activate it. This LT spike typically has a high frequency burst of from two to seven conventional action potentials riding its peak.

Most of our detailed understanding of this LT Ca^{2+} spike derives from *in vitro* slice studies of the mammalian thalamus (e.g., Crunelli et al. 1989; Jahnsen and Llinás 1984a,b; Leresche et al. 1991; McCormick and Feeser 1990). Jahnsen and Llinás (1984a,b) first drew attention to the effect of the LT spike on firing of conventional action potentials, referring to firing at depolarized levels at which the Ca^{2+} conductance was inactivated as the tonic or relay firing mode and to firing at hyperpolarized levels at which LT spikes were activated as the burst mode. Jahnsen and Llinás (1984a,b) further noted that, while in the burst mode, the Ca^{2+} conductance may trigger a complex sequence of other conductances that causes rhythmic LT spiking to persist at 6 Hz, and other studies suggest that the LT spike may oscillate at lower frequencies as well (Curró Dossi et al. 1992; Leresche et al. 1991; McCormick and Pape 1990). These observations have led to suggestions that the response mode reflecting the presence or absence of LT spiking might have dramatic effects on the relay of information from the retina to cortex, from a fairly faithful relay in the tonic mode to significant modification or disruption in the burst mode (Jahnsen and Llinás 1984a,b; Sherman and Koch 1986, 1990). The rhythmic bursting seen in thalamic neurons even suggests the possibility that, once initiated, LT spiking may continue irrespective of retinal input, thereby producing a sort of functional disconnection between retinal afferents and geniculate relay cells. Thus the relay mode of firing provides a faithful transmission to cortex of retinal stimulation, whereas the burst mode indicates that retinal signals are no longer being relayed (Deschênes et al. 1984; Sherman and Koch 1986, 1990; Steriade and Deschênes 1984; Steriade and Llinás 1988). This implies that the unique pattern of the burst provides a positive signal to cortex that is an unambiguous alternative to no firing at all, which could be confused with the relay mode in the absence of visually driven activity (Sherman and Koch 1986, 1990).

The main purpose of this study was to test this general hypothesis regarding the effect on retinogeniculate transmission of LT spiking. Although the presence of LT spiking in thalamic neurons, including those of the lateral geniculate nucleus, has been documented *in vivo* (Deschênes et al. 1984; Hirsch et al. 1983; Hu et al. 1989a,b; Lo et al. 1991), there has been no study of the effects of this on receptive field properties. Therefore we determined the effects of the voltage-dependent Ca^{2+} conductance on responses of geniculate neurons to standard visual stimuli. We used both intracellular and extracellular recording. Our intracellular study (this paper) was done both to document the presence and voltage dependency of LT spiking in response to visual stimuli and also to determine criteria for identification of LT spiking with extracellular recording.

METHODS

Animal preparation and geniculate recording

We performed experiments on adult cats (1.8–3.0 kg) with methods that have been described in detail elsewhere (Bloomfield et al. 1987; Bloomfield and Sherman 1988; Lo et al. 1991) and are only briefly outlined here. For initial surgical preparation, we anesthetized the cats with 2–3% halothane in $\text{N}_2\text{O}-\text{O}_2$ mixed in a 1:1 ratio, and we maintained anesthesia with 0.3–1.0% halothane in a 7:3 mixture of $\text{N}_2\text{O}-\text{O}_2$ throughout the recording session. For paralysis, we administered 5.0 mg gallamine triethiodide followed by 3.6 mg/h of gallamine triethiodide plus 0.7 mg/h of *d*-tubocurarine in 5% lactated Ringer solution. Cats were artificially respired via a tracheal cannula. Rectal temperature, heart rate, and end-tidal CO_2 were monitored and kept within normal physiological limits.

In our anesthetized, paralyzed preparation, we observed electroencephalogram (EEG) activity that is typified by this preparation (Funke and Eysel 1992; Ikeda and Wright 1974); that is, the EEG was usually synchronized in a fashion characteristic of slow-wave sleep, but occasionally this was interrupted by brief periods of spindle activity. We made no attempt to correlate EEG activity with changes of response mode in single neurons.

We mounted the cat in a stereotaxic apparatus and opened the skull to allow recording from the lateral geniculate nucleus. A plastic well was built around the craniotomy, and the chamber was sealed with agar and wax to improve stability during recording. We inserted a pair of bipolar stimulating electrodes into the brain so that the tips straddled the optic chiasm. We applied pulses (0.1-ms duration, 100–500 μA , ≤ 1 Hz) across the chiasm electrodes to activate geniculate neurons orthodromically.

The pupils were dilated, accommodation was blocked pharmacologically, and the corneas were protected with zero-power contact lenses that contained a 3-mm-diameter artificial pupil. We used a fiber optic light source to plot and project retinal landmarks, including the area centralis, onto a tangent screen. Spectacle lenses focused the eyes onto the same tangent screen or onto an electronic display monitor placed in front of the cat, 28.5 cm from the nodal points of the eyes.

Single neurons in the A-laminae of the lateral geniculate nucleus were recorded intracellularly by the use of fine-tipped micropipettes filled with 4M KAc. We pulled the electrode to an initial impedance of 40–50 M Ω and then beveled the tip to a final impedance of 20–30 M Ω . Requirements for acceptable intracellular impalement included a DC drop in resting potential to -50 mV or more negative, an action potential amplitude of ≥ 40 mV, and a neuronal input resistance ≥ 10 M Ω .

We amplified neuronal activity through a high-impedance amplifier equipped with a bridge and current injection circuitry (Axon Instruments). We displayed all recordings on an oscilloscope, fed them to an audio monitor, and stored them on an eight-channel FM tape recorder interfaced with a computer for off-line analysis. Action potentials were led through a window discriminator to a computer for off-line analysis.

Visual stimulation and geniculate cell classification

For the initial evaluation of neuronal responses, visual stimuli were presented on a tangent screen using a hand-held projector. We used flashed spots of light to determine ocular dominance, receptive field location, receptive field size, and off- or on-center type. We then replaced the tangent screen with a display monitor to present vertically oriented, sine-wave grating stimuli. The gratings were produced with an image generator (Innisfree) controlled by a computer. The gratings had a mean luminance of 30 cd/m 2 and could be drifted or counterphased modulated. Other stimulus

parameters such as spatial frequency, contrast, and temporal frequency were varied independently.

We classified all but one geniculate neuron as X or Y by the use of a standard battery of tests. This included linearity of spatial summation in response to grating stimuli; receptive field center size; response latency to electrical stimulation of optic chiasm; and response to a large, fast-moving stimulus of high contrast to activate the surround (i.e., dark for an on-center cell). One neuron for which we were unable to complete a full battery of tests was placed into an unclassified category.

Intracellular responses to drifting gratings

Typically, we presented drifting sine-wave gratings of various temporal (1–25 Hz) and spatial frequencies (0–1.0 cycle/deg) at a contrast of 0.5. We stored the spike arrival times of the responses to visual stimuli with a resolution of 0.1 ms. Responses were evalu-

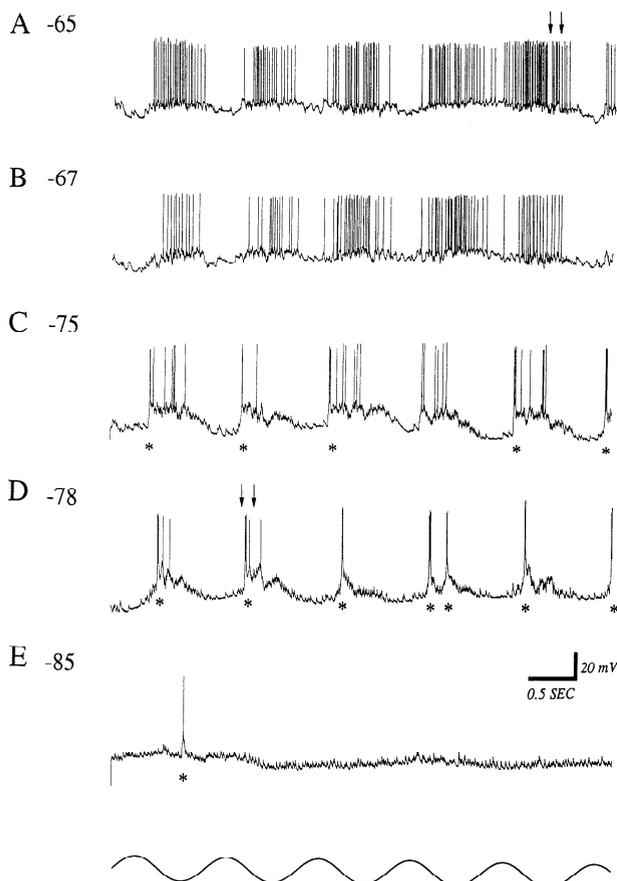


FIG. 1. Intracellular records showing responses of a geniculate X cell to a drifting sine-wave grating at different membrane potentials. *Bottom trace*: sinusoidal contrast changes presented by drifting grating. Each LT spike is depicted by an asterisk below the trace. *A*: responses at the resting membrane potential, which was -65 mV. Trace segment between 2 arrows is shown at an expanded time base in Fig. 3*A*, *top*. *B*: responses at a membrane potential of -67 mV. *C*: responses at a membrane potential of -75 mV. *D*: responses at a membrane potential of -78 mV. Trace segment between 2 arrows is shown at an expanded time base in Fig. 3*A*, *bottom*. *E*: responses at most hyperpolarized membrane potential. Here, cell was sufficiently hyperpolarized that responses of any type were rare. In *A* and *B*, no LT spikes were seen and response was purely tonic. LT spikes were often activated at more hyperpolarized potentials (*C* and *D*). Note that, when they occur, they are always the 1st response evoked, and tonic responses may occur later. More than 1 LT spike may rarely be evoked during a stimulus cycle (e.g., 4th cycle of *D*).

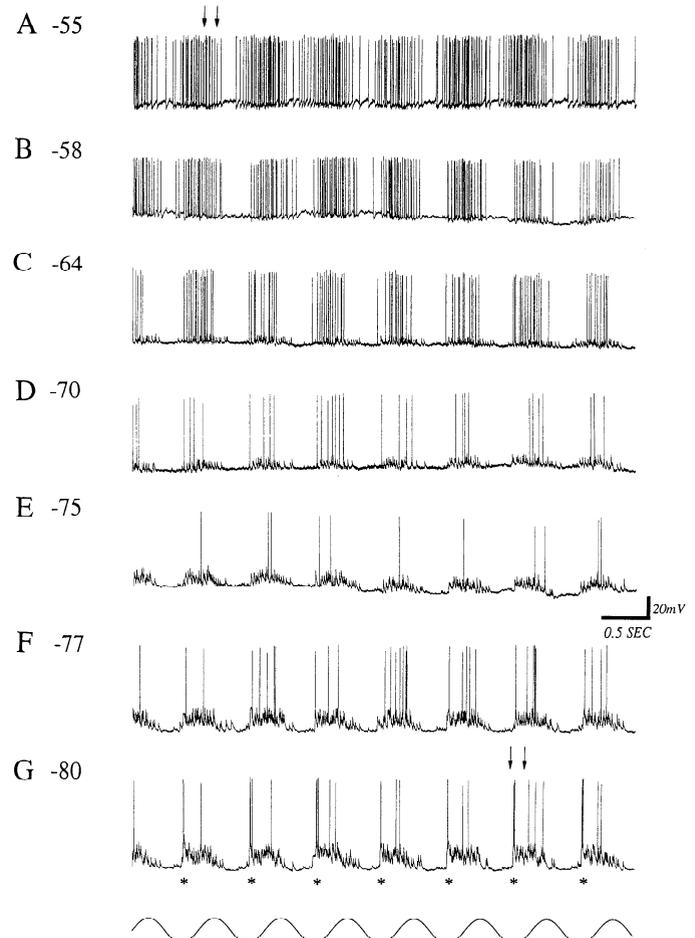


FIG. 2. Intracellular records showing responses of a geniculate Y cell to a drifting sine-wave grating at different membrane potentials; same conventions as Fig. 1, and cell's resting membrane potential was -67 mV. *A*: responses at a membrane potential of -55 mV. Trace segment between 2 arrows is shown at an expanded time base in Fig. 3*B*, *top*. *B*: responses at a membrane potential of -58 mV. *C*: responses at a membrane potential of -64 mV. *D*: responses at a membrane potential of -70 mV. *E*: responses at a membrane potential of -75 mV. *F*: responses at a membrane potential of -77 mV. *G*: responses at a membrane potential of -80 mV. Trace segment between 2 arrows is shown at an expanded time base in Fig. 3*B*, *bottom*. LT spikes were not evident at depolarized membrane potentials (*A–F*), but did appear at most hyperpolarized level tested (*G*), and they are clearly evoked by the stimulus. As is the case for the X cell in Fig. 1, when these LT spikes occur, they are always the 1st response evoked, and tonic responses may occur later.

ated by computing the Fourier components from the averaged response histogram; usually the fundamental component (F1) was used as the response measure, although the second harmonic (F2) component was occasionally used. During visual stimulation, we varied membrane voltage (V_m) by direct current injection through the recording electrode. For each parameter tested, we maintained a particular V_m for a period of 20–40 s or 30–80 stimulus cycles. Equivalent epochs in which a uniform luminance equal to the mean luminance of the gratings also was presented in order to obtain a measure of spontaneous activity. For some neurons (2 X and 2 Y) we were able to construct spatial frequency tuning functions at several different membrane potentials. However, for the majority of neurons in our sample, grating parameters were held constant at or near optimal values of spatial and temporal frequency while we varied membrane voltage.

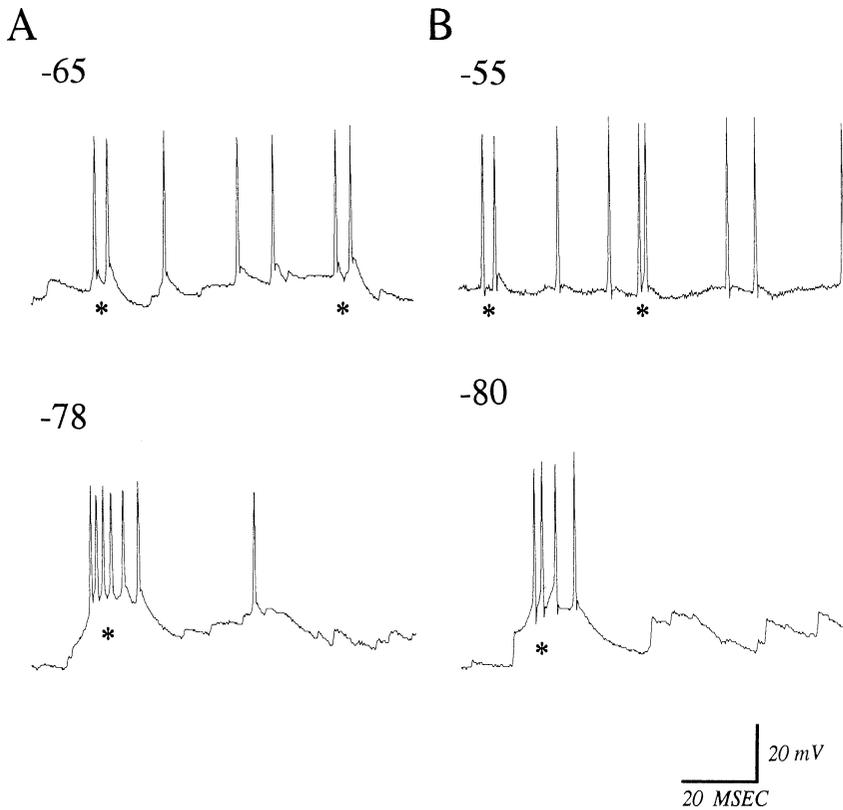


FIG. 3. Intracellular records showing low- and high-threshold burst responses (LT and HT bursts) of a geniculate X and Y cell. LT burst derives from an LT spike, and HT burst is part of tonic response. Traces are expanded sequences from Figs. 1 and 2. *A*: HT bursts (asterisks, *top trace*) and LT bursts (asterisk, *bottom trace*) from an X cell. *B*: HT bursts (asterisks, *top trace*) and LT bursts (asterisk, *bottom trace*) from a Y cell. Numbers reflect membrane voltage in mV.

RESULTS

A main purpose of these experiments was to determine the effects of LT Ca^{2+} spikes on responses of cells in the cat's lateral geniculate nucleus to visual stimuli. This re-

quired in vivo recording techniques for visual response profiles and intracellular recording to verify the presence of LT spikes. The intracellular recording allowed us to verify both the presence of the large triangular depolarization due to the Ca^{2+} conductance and its voltage dependency (see IN-

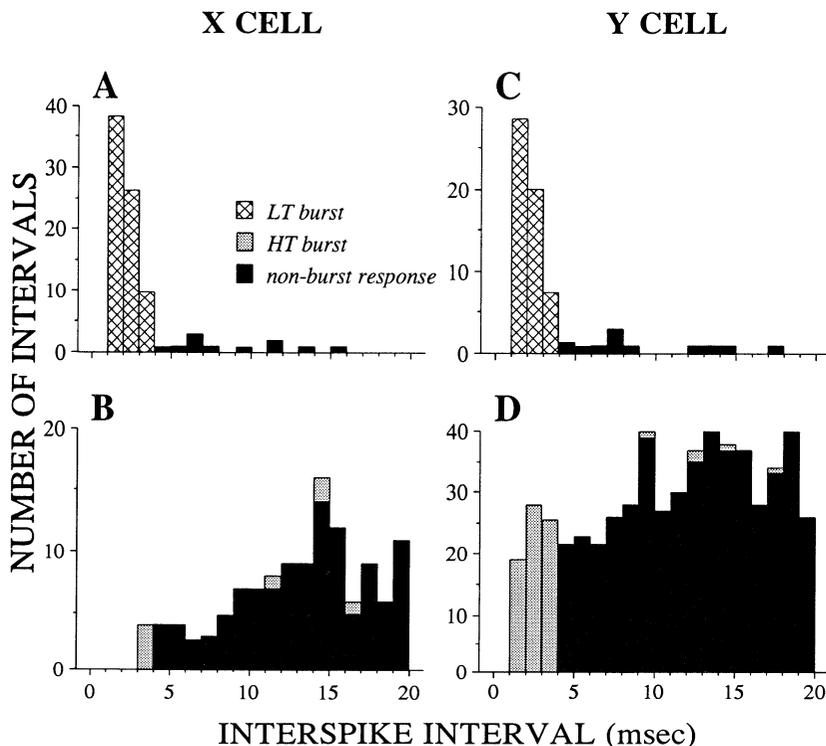


FIG. 4. Pattern of interspike intervals at different membrane potentials for a geniculate X and Y cell. All intervals ≤ 20 ms were plotted in these histograms. *A*: intervals at hyperpolarized membrane potential for X cell. Many LT burst and no HT bursts were seen. *B*: intervals at depolarized membrane potential for X cell. Many HT burst and no LT bursts were seen. *C*: intervals at hyperpolarized membrane potential for Y cell. Many LT burst and no HT bursts were seen. *D*: intervals at depolarized membrane potential for Y cell. Many HT burst and no LT bursts were seen.

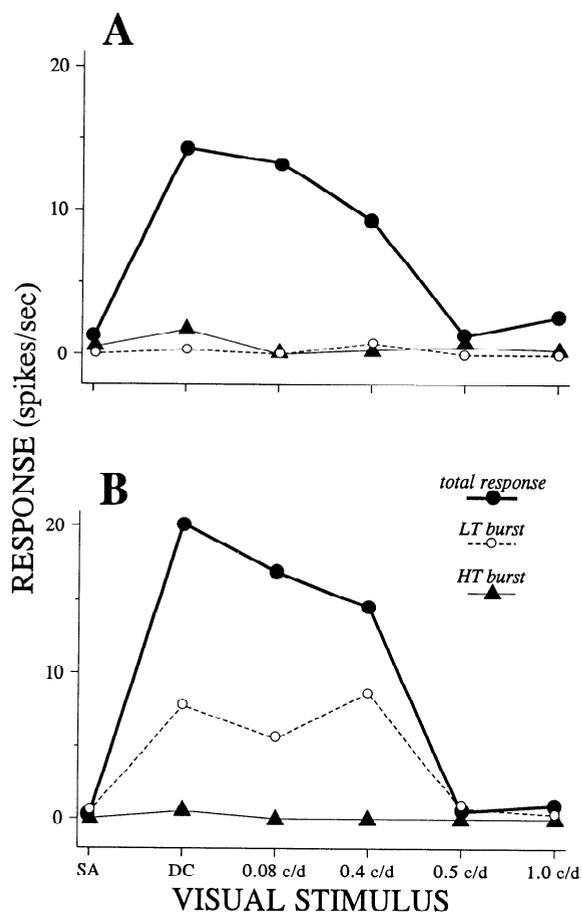


FIG. 5. Spatial tuning functions for a geniculate X cell at different membrane potentials. Plotted are fundamental Fourier amplitudes of total response as well as LT and HT bursts contained within total response. Responses were evoked by a grating drifted at 2 Hz and at different spatial frequencies as indicated; also shown are responses to whole field sinusoidal flicker at 2 Hz (DC) and to a blank screen of the same average luminance (SA), which provides a measure of spontaneous activity. *A*: tuning at a membrane potential of -62 mV. *B*: tuning at a membrane potential of -80 mV.

INTRODUCTION). This voltage dependency required that we monitor responses of geniculate neurons at different membrane potentials (see DISCUSSION). To achieve this required lengthy, stable intracellular recording. Although we recorded intracellularly from numerous cells, we often failed to maintain stable recording long enough to assess the voltage dependency of responses to visual stimulation. Thus the data described below are taken from the 14 geniculate cells (6 X, 7 Y, and 1 unclassified) for which voltage-dependent responses were determined. Every one of these cells exhibited LT spikes.

It is interesting in the context of the voltage dependency of LT spikes that we did not observe large (≥ 10 mV), spontaneous shifts in membrane potential. We did see spontaneous shifts, which were probably postsynaptic potentials, but these were much smaller. The implication of this is considered in more detail in the following paper (Guido et al. 1992).

Figures 1 and 2 illustrate many of the basic results of our study. These show for an X and a Y cell (Figs. 1 and 2,

respectively) the effects of membrane potential on responses to a drifting sine-wave grating. At the more depolarized levels (Figs. 1, *A* and *B*, and 2, *A-F*), there is no evidence of LT spiking, and the responses are in the form of a fairly continuous stream of action potentials to each excitatory phase of the stimulus. In keeping with prior descriptions of these responses, we refer to this response component as the tonic response (Jahnsen and Llinás 1984a,b). As expected, the responses are usually more vigorous at the more depolarized levels. At more hyperpolarized levels (Figs. 1, *C-E*, and 2*G*), LT spiking occurs. Because this almost always entails a burst of conventional action potentials riding the crest of the LT spike, we refer to this response component as the LT burst (Jahnsen and Llinás 1984a,b).

Three features characterize these LT responses. First, LT bursts occur in phase with the visual stimulus and, once initiated, do not occur spontaneously at a rate independent of the visual stimulus. LT spiking is thus visually driven, and the appearance of LT bursts does not necessarily signify a decoupling of geniculate neurons from their retinal inputs (see INTRODUCTION). Indeed, because LT spikes are faithfully activated at hyperpolarized levels and each represents a large depolarization that helps to generate the burst of action potentials, this may be viewed as a “boosting” or amplification that enables a hyperpolarized cell to respond more strongly to visual stimuli. Second, if any response occurs to a cycle of the visual stimulus at membrane levels sufficiently hyperpolarized to deactivate LT spiking, at least one LT spike occurs in the response. Rarely and only at the lowest temporal frequencies (i.e., ~ 1 Hz), there may be more than one LT spike in a response cycle (Fig. 1*D*). LT bursts also may be mixed in a response cycle with a tonic response component, although often LT bursts are the only response seen. A purely tonic response was never seen at these membrane levels. Third, when a stimulus cycle evoked a mixture of LT bursts and tonic responses, the LT spike always occurred first. These three observations are considered in more detail below.

Criteria for LT burst responses

Although we found it a straightforward matter with intracellular recording to identify the presence of an LT spike and its associated burst response, principally because of the large underlying depolarization and voltage dependency, we sought to characterize this response further in terms solely of the pattern of action potentials. This pattern represents the actual signal reaching cortex and is thus important in understanding the effective retinogeniculate transmission during the burst response mode. Another value of such a characterization is that it enables the use of extracellular recording techniques to be applied to the study of LT spiking (see following paper, Guido et al. 1992). Such attempts in the past have focused largely on interspike intervals as well as other parameters of the response profile (Lo et al. 1991; McCarley et al. 1983; McCormick 1992; Steriade et al. 1989) to characterize LT spiking, but this has not yet been done in the context of visual stimulation. Often an interspike interval of ≤ 4 ms or a firing rate ≥ 250 Hz has

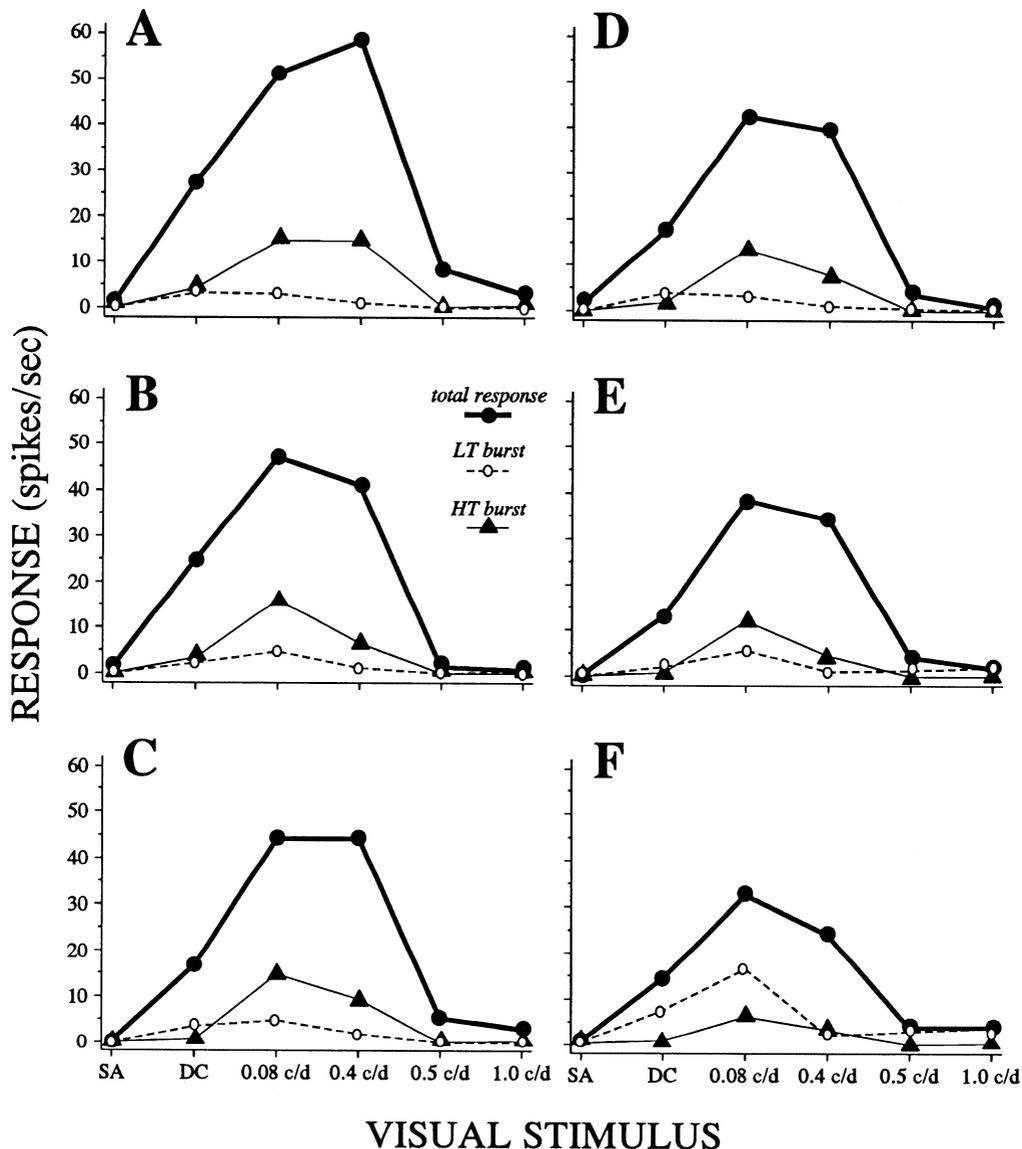


FIG. 6. Spatial tuning functions for a geniculate Y cell at different membrane potentials; conventions and stimuli as in Fig. 5. *A*: tuning at a membrane potential of -65 mV. *B*: tuning at a membrane potential of -67 mV. *C*: tuning at a membrane potential of -70 mV. *D*: tuning at a membrane potential of -72 mV. *E*: tuning at a membrane potential of -75 mV. *F*: tuning at a membrane potential of -80 mV.

been attributed to LT bursts (Lo et al. 1991; McCarley et al. 1983; McCormick 1992; Steriade et al. 1989). However, it may be obvious from Figs. 1 and 2 that, at least for these geniculate neurons, a criterion based on interspike interval alone is insufficient for the identification of LT bursts; that is, during depolarized membrane potentials at which the LT spike is inactivated, high-frequency discharges with interspike intervals ≤ 4 ms can be elicited by visual stimuli. This is shown more clearly in Fig. 3.

For the purposes of exposition only, we shall refer to all clusters of action potentials with interspike intervals ≤ 4 ms as "bursts," but we distinguish between low threshold (LT) bursts and high threshold (HT) bursts: the former derive from LT spikes and thus have a low threshold of activation, whereas the latter derive conventionally from strong post-

synaptic depolarization and thus display the higher threshold of action potentials. To determine the pattern of responses that distinguish LT from HT bursts and any other responses, we examined in detail intracellular records from four X and four Y cells during responses to the drifting sine-wave gratings and also during spontaneous activity. We varied the spatiotemporal parameters of the gratings (≤ 1.0 cycles/deg and ≤ 8 Hz; responses to higher temporal frequencies are described below), and we varied membrane potential to include periods when LT spiking was inactivated and deinactivated. Overall, we sampled 1,817 burst epochs (i.e., responses with interspike intervals ≤ 4 ms) chosen at random and representing a wide range of membrane potentials and spatiotemporal variables of visual stimulation. We broke these down, on the basis of membrane

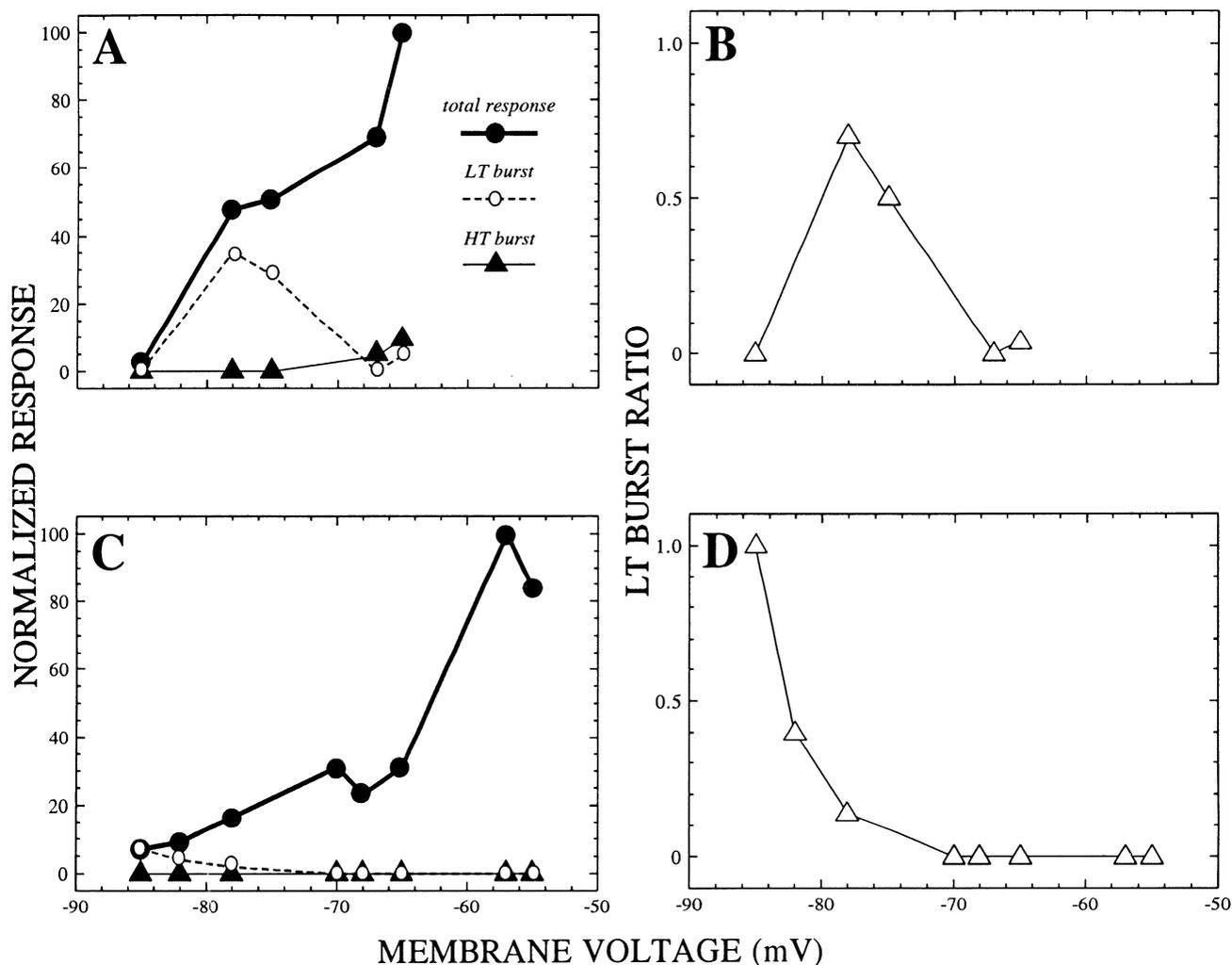


FIG. 7. Effects of membrane voltage on LT and HT bursting for 2 geniculate X cells. *A*: normalized responses as a function of membrane potential for 1 X cell. Total response and burst components were normalized against the peak fundamental Fourier amplitude of the total response, the value of which was set to 100. *B*: LT burst ratio as a function of membrane potential for the X cell illustrated in *A*. LT burst ratio was computed by dividing the fundamental Fourier amplitude of the LT burst by the sum of fundamental amplitudes of LT burst and tonic response components (see text for details). *C*: normalized responses as a function of membrane potential for another X cell. *D*: LT burst ratio as a function of membrane potential for the X cell illustrated in *C*.

voltage and the presence or absence of the characteristic depolarization associated with the Ca^{2+} conductance, into 282 LT bursts and 1,535 HT bursts.

We found that each of the 282 LT bursts (100%) was characterized by a preceding period of no action potentials that lasted ≥ 100 ms, whereas only 22 of the 1,535 HT bursts (1.4%) exhibited a preceding silent period of ≥ 100 ms. However, because our highest temporal frequency of stimulation was 8 Hz, the cycle time (125 ms) exceeded the preceding silent period for LT spiking, and we might ask what happens with faster temporal rates. As noted below and in the following paper (Guido et al. 1992), many of these geniculate cells exhibit LT spiking in response to gratings drifted at 16 Hz, but little or none was detected at 32 Hz. At 16 Hz, we often saw LT spikes occurring with silent periods equal to the cycle time (~ 60 ms), although such shorter silent periods were never exhibited at lower rates of

stimulation. If we thus adopted a criterion of a 50-ms silent period for responses to 16-Hz stimulation, we still observed that none of the LT bursts occurred after a shorter silent period, whereas 29 of the HT bursts (1.9%) exhibited a preceding silent period of ≥ 50 ms.

In summary, we find that, at frequencies of visual stimulation ≤ 8 Hz, the criterion of a preceding silent period of 100 ms dictates that we would never mistake an LT for an HT burst, and only 1.4% of our HT bursts would be mistaken for LT bursts; at higher temporal frequencies, the reduction of the silent period to 50 ms still dictates that we would not mistake an LT for an HT burst, and the rate of misidentifying HT for LT bursts rises to only 1.9%. We therefore conclude that the presence of an LT spike can be determined with great reliability ($>98\%$) on the basis of the temporal pattern of action potentials: if a burst of action potentials occurs (i.e., several action potentials with interspike inter-

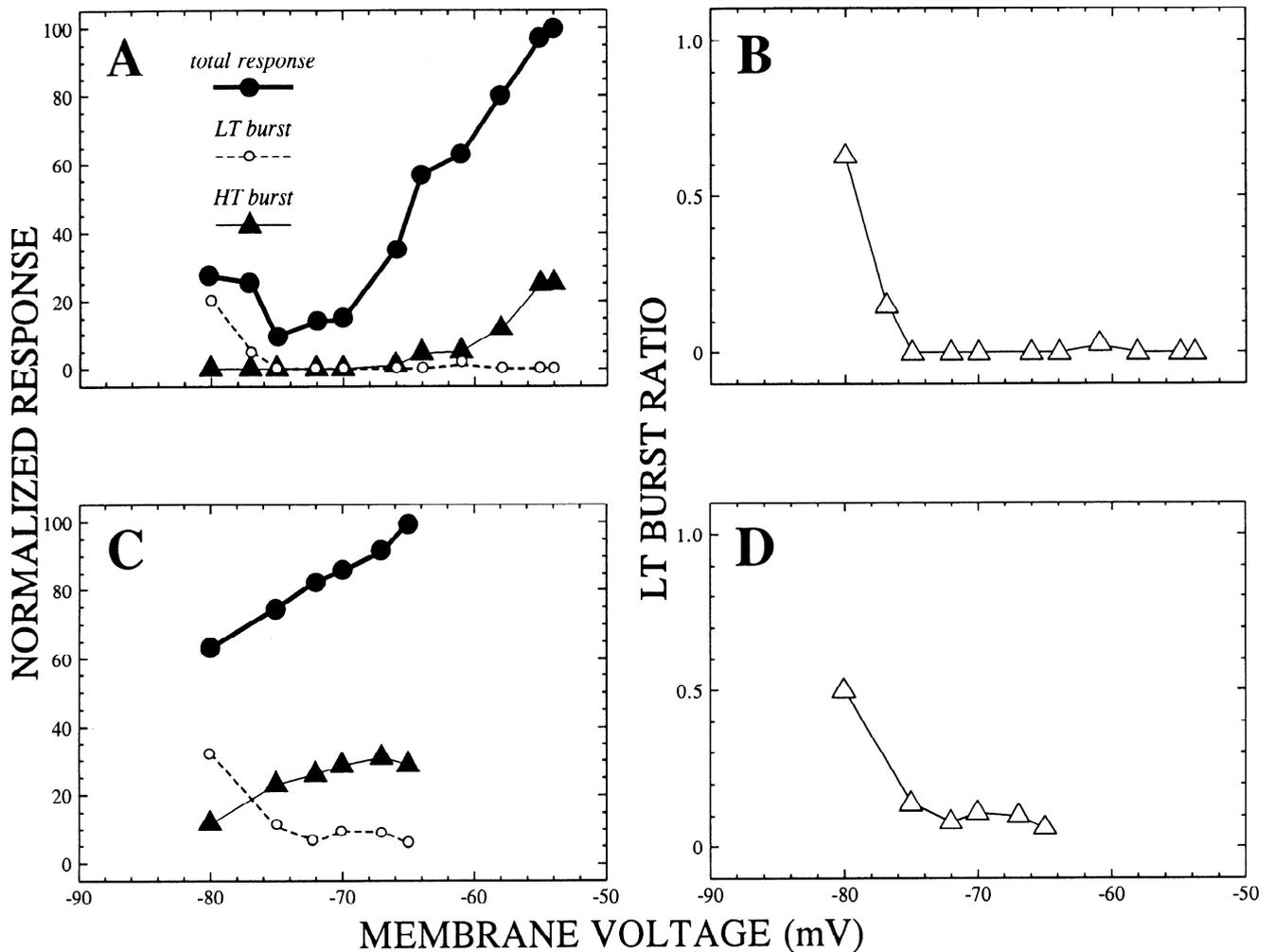


FIG. 8. Effects of membrane voltage on LT and HT bursting for 2 geniculate Y cells; conventions as in Fig. 7. *A*: normalized responses as a function of membrane potential for 1 Y cell. *B*: LT burst ratio as a function of membrane potential for the Y cell illustrated in *A*. *C*: normalized responses as a function of membrane potential for another Y cell. *D*: LT burst ratio as a function of membrane potential for the Y cell illustrated in *C*.

vals of ≤ 4 ms) after a period without action potentials lasting ≥ 100 ms (or 50 ms during responses to high temporal frequency visual stimulation), it is an LT burst; if the burst occurs with a preceding silent period of ≤ 100 ms (or 50 ms), it is an HT burst. As noted in the DISCUSSION, these empirically determined differential patterns of response for LT and HT bursts are consistent with other properties of LT and HT bursting.

Figure 4 further demonstrates these criteria for an X and a Y cell held at different membrane potentials by showing the interspike intervals seen in response to a grating drifting at near optimal spatial and temporal frequencies. For each cell, at the more hyperpolarized potential at which LT spiking was evident, no HT bursts were seen, and conversely, at the more depolarized level at which the LT spike was inactivated, the only bursts present were HT bursts. Note that action potentials in the LT bursts always had interspike intervals of ≤ 4 or > 20 ms, because the first action potential of the burst occurred only after a ≥ 100 -ms silent period. However, a few of the interspike intervals for the HT bursts occasionally fell between 4 and 20 ms, because the first

action potential of a burst may occur shortly after an individual action potential.

Voltage dependency of LT and HT bursts

As noted above, Figs. 1–3 suggest that LT and HT bursts may also differ in regard to requirements of membrane voltage, because the former occurs chiefly when the cell is relatively hyperpolarized, whereas the latter is more common during depolarization. In further experiments performed on other X and Y cells, we found this distinction to hold. For instance, Figs. 5 and 6 show the fundamental Fourier response amplitudes of an X and a Y cell to a grating drifted at different spatial frequencies. The X cell shows very few HT bursts, and these occur essentially only while the cell was relatively depolarized (Fig. 5*A*), whereas LT spiking occurs only during hyperpolarization (Fig. 5*B*). The Y cell shows a similar pattern: at more depolarized levels, HT bursts are more evident than are LT bursts (Fig. 6, *A–D*), whereas at the most hyperpolarized level, LT bursts are more prevalent (Fig. 6*F*). Note also that the spatial tuning

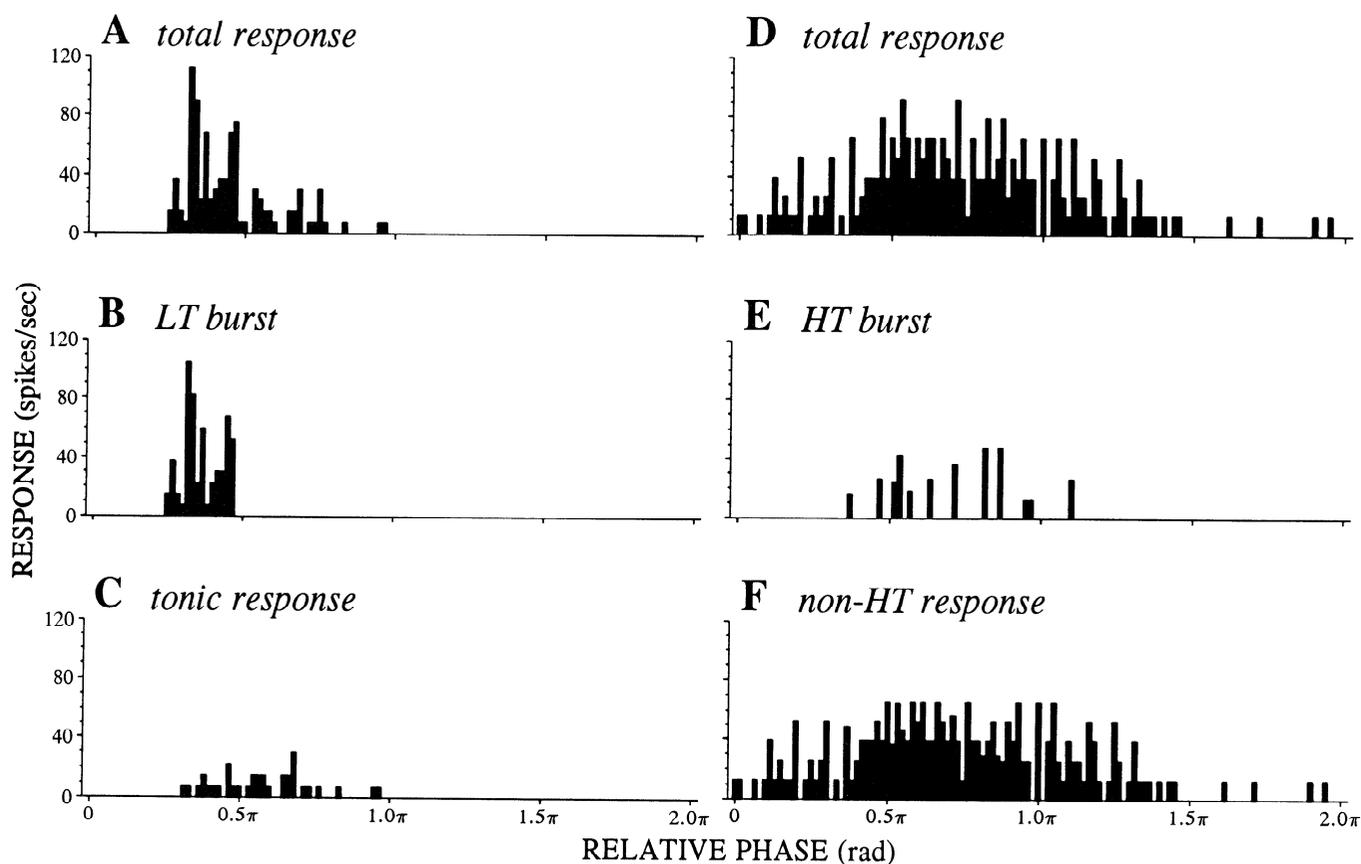


FIG. 9. Typical response histograms to 1 cycle of a drifting grating for a geniculate X cell at different membrane potentials. Response is shown separately for total response, LT burst, tonic response, HT burst, and non-HT response. *A-C*: responses at -80 mV. LT spiking was evident, and no HT bursts were seen. *D-F*: responses at -65 mV. No LT spiking was evident, and HT bursts were seen.

is roughly similar at all membrane potentials for the total responses, the HT bursts, and the LT bursts.

The voltage dependency of these LT and HT bursts is further illustrated for additional X and Y cells in Figs. 7 and 8. These figures reflect responses to a grating drifting at near optimal spatial and temporal frequencies, and the responses shown reflect the fundamental Fourier amplitudes. The left panels of each illustration show the normalized fundamental Fourier amplitudes as a function of membrane voltage, and these are shown separately for the total response, for only the LT bursts, and for only the HT bursts. For the burst components, we simply used the above-mentioned criteria to extract from the overall responses the portion due to LT or HT bursts. As expected, the overall response generally increases with depolarization. Generally, LT bursts are seen at more hyperpolarized levels, and HT bursts at more depolarized ones.

The Y cell shown in Fig. 8*A* illustrates an interesting phenomenon occasionally seen. Its overall response does not monotonically decline with hyperpolarization, because at more hyperpolarized levels, the LT bursting that kicks in more than compensates for the reduction in tonic response component. Note also from Fig. 7*A* that it is possible to hyperpolarize a cell past the point at which LT spiking may be evoked. No response was seen at this most hyperpolarized level, presumably because the depolarization due to the visual stimulus was insufficient to activate the LT con-

ductance from its severe level of hyperpolarization. Although we can refer to the LT spike as "low threshold," it nonetheless does have a threshold that must be reached.

The right panels of Figs. 7 and 8 show for each cell the effect of membrane potential on the relative amplitude of the LT burst, or the LT burst ratio. This ratio was computed as follows. By using the above criteria to detect the burst of action potentials caused by LT spiking, we divided the total response into LT burst and tonic components. We then divided the LT burst component by the sum of these two components, and this value represents the LT burst ratio. Plots of this ratio versus membrane potential not only emphasize the point that LT spiking occurs only at the most hyperpolarized membrane potentials, but they also show that, at these potentials, most, and occasionally all, of the cell's response is contained in LT bursts.

Linearity of response for LT spiking

Figures 9 and 10 illustrate the difference in response profiles between LT and HT bursts. As noted above, we used our criteria involving the pattern of action potentials to analyze separately the burst response and remaining components. At more hyperpolarized levels (Figs. 9, *A-C*, and 10, *A-C*), a major component of the total response is due to LT bursts, and no HT bursts were seen. When the response is broken down into the LT burst (Figs. 9*B* and 10*B*) and

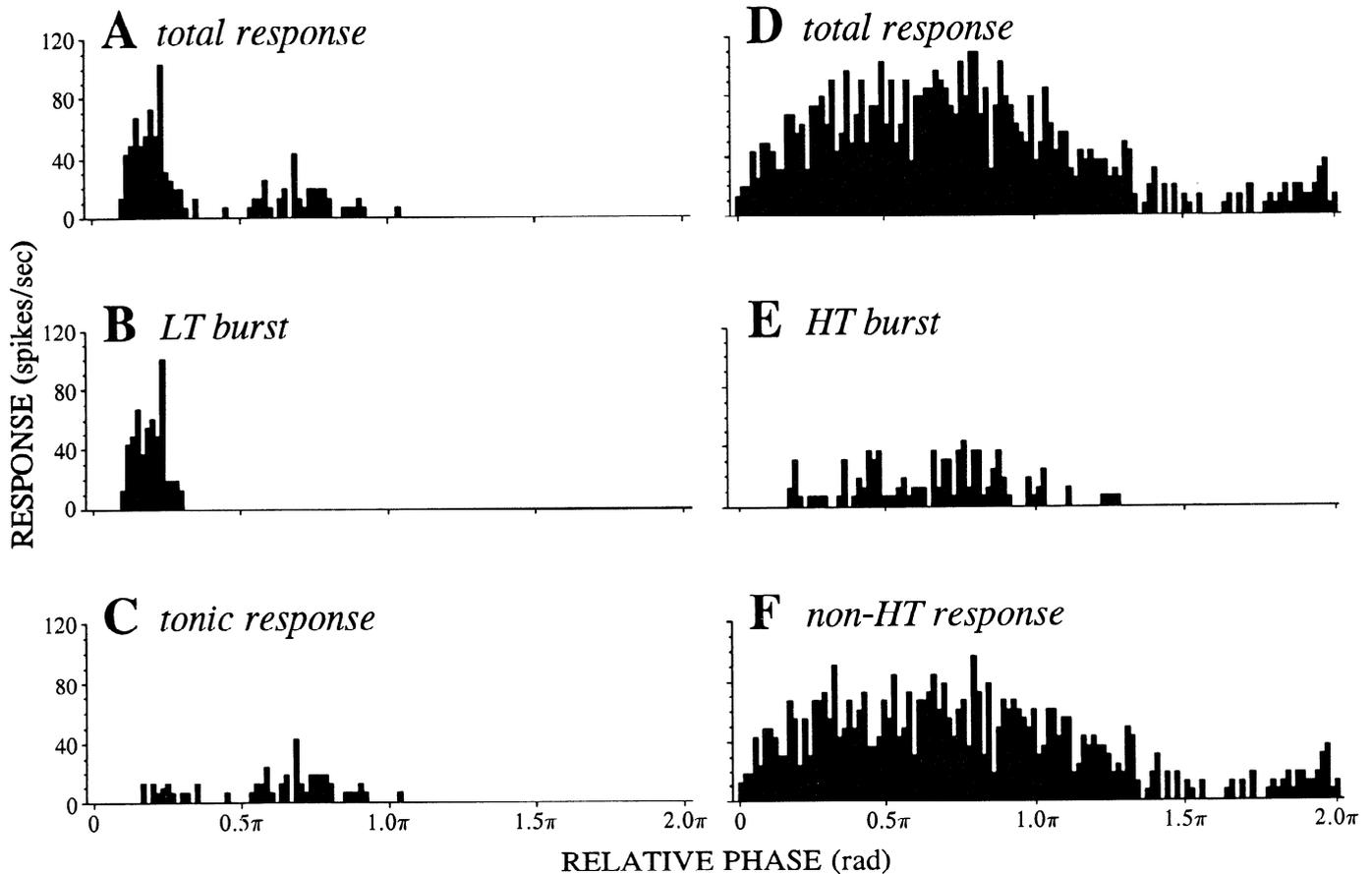


FIG. 10. Typical response histograms to 1 cycle of a drifting grating for a geniculate Y cell at different membrane potentials; conventions as in Fig. 9. *A-C*: responses at -80 mV. LT spiking was evident, and no HT bursts were seen. *D-F*: responses at -55 mV. No LT spiking was evident, and HT bursts were seen.

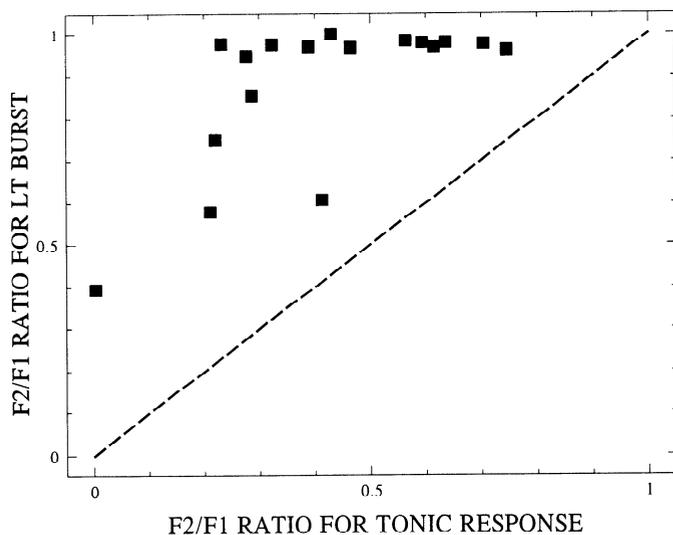


FIG. 11. Scatter plot showing increased nonlinearity for LT burst compared with the tonic response component for 8 geniculate neurons (4 X and 4 Y) recorded at membrane potentials at which LT spiking occurred. More than 8 points are shown because the cellular responses to >1 stimulus condition were analyzed, and each stimulus/response measure is plotted (see text for details). Nonlinearity is estimated as the ratio of the second harmonic to the fundamental Fourier amplitude of the response ($F2/F1$). Line of slope 1 is also shown. Note that each point lies above this line, meaning that the LT burst contains more nonlinear distortion than does the tonic response component.

tonic response (Figs. 9C and 10C) components, it is clear that they differ in structure. The LT burst occurs earlier and seems nonlinear, because it conspicuously fails to follow the sinusoidal shape of the contrast change presented by the drifting grating. The tonic response component occurs later and is more linear. Thus much of the nonlinearity seen in the overall response (Figs. 9A and 10A) is due to the LT spiking. At more depolarized levels (Figs. 9, D-F, and 10, D-F), no LT spikes were seen, and all of the bursting was in the form of HT bursts. In contrast to the situation with more hyperpolarized levels, the HT bursts (Figs. 9E and 10E) are neither noticeably shifted in time nor less linear than are the remaining, nonburst responses (Figs. 9F and 10F).

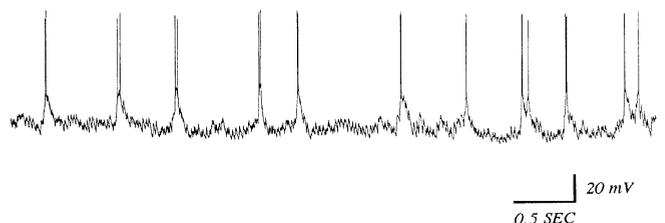


FIG. 12. LT spiking present during spontaneous activity in a geniculate X cell at a membrane potential of -75 mV; same cell as in Fig. 1.

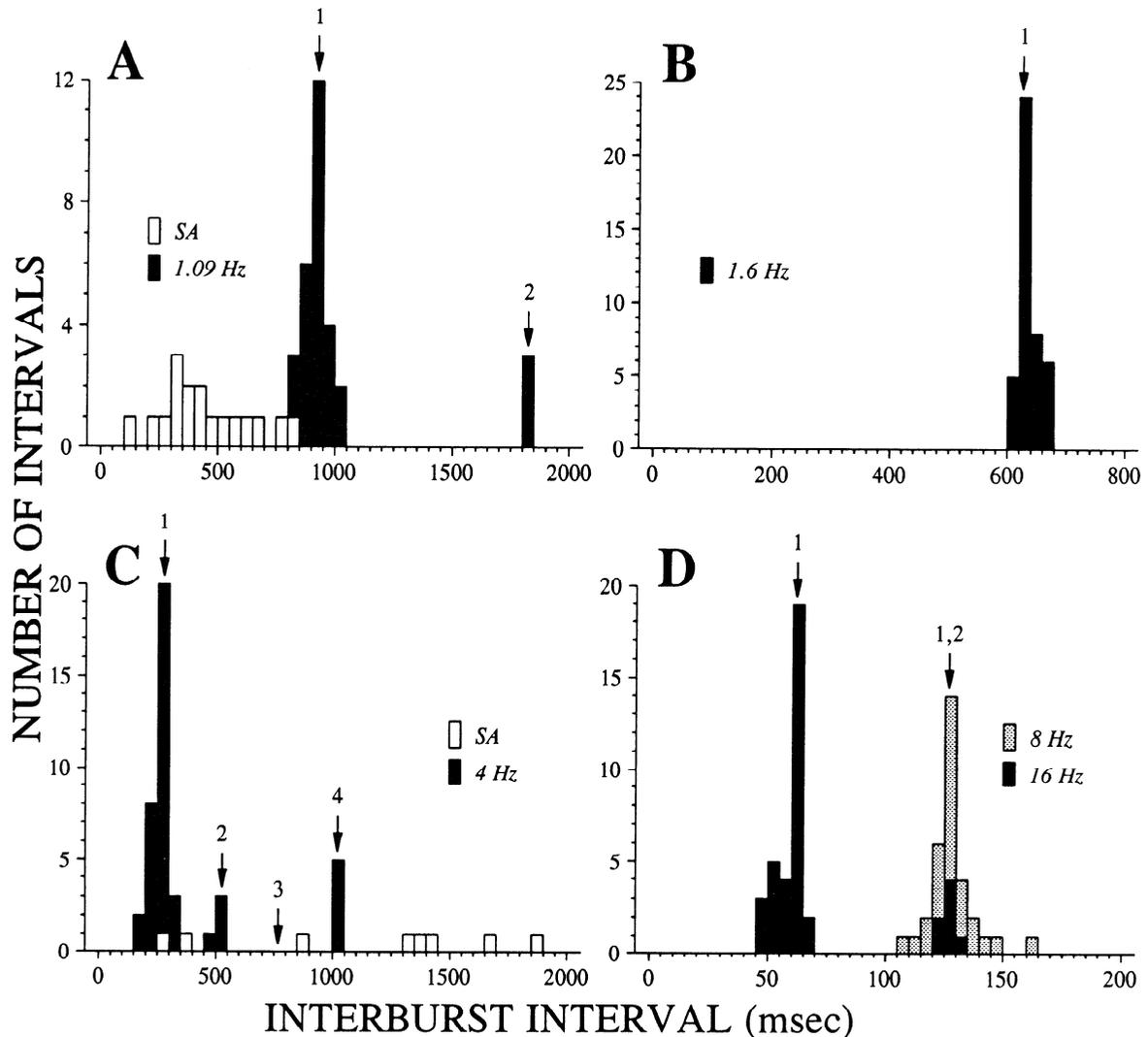


FIG. 13. Histograms of intervals between LT bursts (or interburst intervals) in activity recorded from 4 geniculate neurons at membrane potentials at which LT spiking occurred. These were computed by measuring the time intervals between the 1st action potentials of each LT burst. Shown are responses during spontaneous activity (SA) and those stimulated by a grating drifted at varying temporal frequencies as indicated. Numbered arrows indicate multiples of the stimulus cycle time. *A*: responses of an X cell. Note that, during spontaneous activity, there is no periodicity of intervals, but when stimulated with a grating, intervals peak mostly around the stimulus cycle time with a secondary peak near twice the cycle time. Secondary peak presumably reflects a preceding stimulus cycle that failed to evoke a response. *B*: responses of another X cell to a drifting grating. All intervals peak around the stimulus cycle time. *C*: responses of a Y cell. As in *A*, this cell shows no clustering of intervals during spontaneous activity, but intervals occur mainly at the stimulus cycle time with subsidiary peaks at integer multiples of the cycle time. *D*: responses of another Y cell to gratings drifted at 2 temporal frequencies. Again, most intervals cluster around the stimulus cycle time.

Figures 9 and 10 indicate that the LT burst is less linear than the tonic response. Figure 11 documents this point more systematically for eight geniculate neurons (4 X and 4 Y), each recorded at a membrane potential at which LT spiking was evident. More than eight points are shown because data were obtained in response to several spatial frequencies of the drifting grating, and each point represents the response to a cell at each frequency. We measured both the fundamental and second Fourier harmonic response component (F1 and F2, respectively). We used these for each cell at each spatial frequency to compute the F2-to-F1 ratio separately for the burst and tonic response components. In every case, this ratio was higher for the LT burst

than for the tonic response component. Because the F1 Fourier amplitude represents the linear response, and the F2 component is a limited measure of response nonlinearity, this indicates that bursting contributes relatively more to nonlinear than to linear responses of the cell. Therefore, although the LT Ca^{2+} conductance offers the cell the opportunity to respond strongly to visual stimuli while hyperpolarized, it does so at the expense of response linearity (see DISCUSSION).

Temporal properties of LT spiking

TEMPORAL TUNING. Our data indicate that LT spiking can respond synchronously to visual stimuli (see above). This

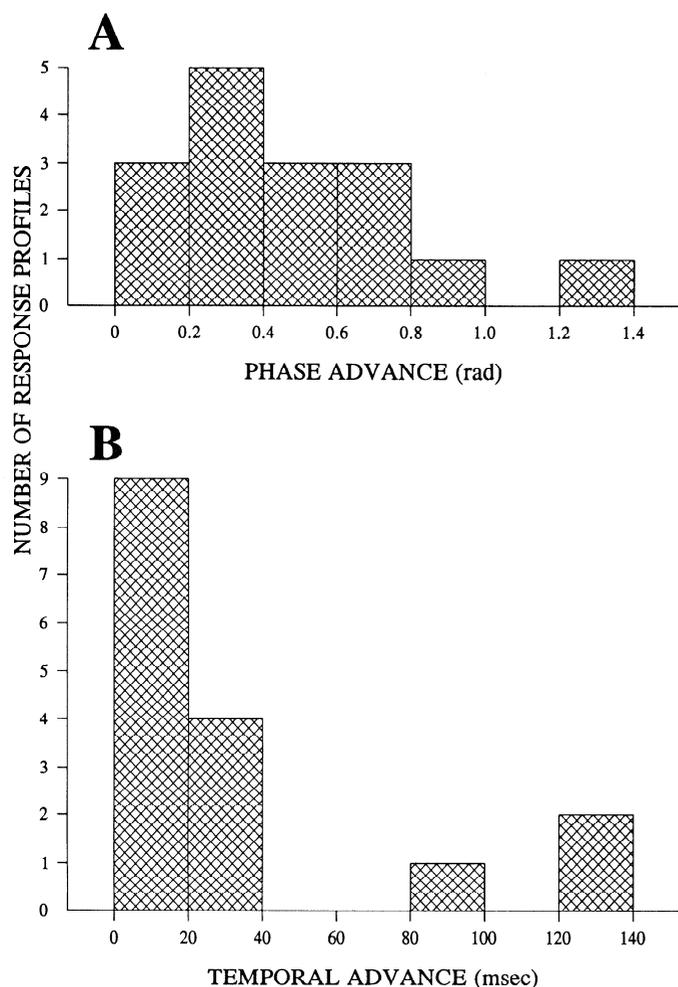


FIG. 14. Relative timing of LT burst and tonic response components in response to drifting gratings. Data are from 6 cells (2 X and 4 Y), but each was recorded at 2 or 3 different membrane potentials at which LT spiking occurred to produce the 16 epochs depicted here. *A*: phase advance of the LT burst relative to the tonic response component. *B*: temporal advance of the LT burst relative to the tonic response component.

point is further illustrated by examining LT spiking evoked by different rates of temporal stimulation. Although we did not systematically construct complete temporal tuning functions during intracellular recording (for examples of this from extracellular recording, see the following paper, Guido et al. 1992), we noted that cells faithfully synchronized their LT spiking to the different visual stimulus rates.

Such synchrony was never evident during spontaneous activity, an example of which is shown in Fig. 12. Here LT spikes occur without a clearly fixed interval between them. This is shown more systematically in Fig. 13 for four different cells held at a membrane potential at which LT spiking occurred. Here, the intervals between LT spikes are shown as a function of the response. During spontaneous activity, cells never showed fixed intervals between LT spikes (Fig. 13, *A* and *C*). However, at different temporal frequencies of stimulation by drifting gratings, the intervals between LT spikes fall into narrowly demarcated clusters at the stimulation frequency or a harmonic thereof (Fig. 13, *A-D*), and the intervals can shift within a cell to reflect a new stimulation frequency (Fig. 13 *D*).

PHASE ADVANCE OF LT BURSTS. As we have noted above in reference to Figs. 1, 2, 9, and 10, the LT burst seems to be elicited earlier than the tonic response. We investigated this further by analyzing the responses to drifting gratings at near optimal spatial and temporal frequencies from six geniculate cells, two X and four Y. Sixteen response epochs were obtained from these six cells by analyzing responses from each at several different membrane potentials for which LT spiking occurred. These responses were separated into LT burst and tonic components according to the criteria defined above; after separation, we computed the F1 of each component plus its phase value. We determined the relative phase advance for the LT bursts by subtracting its phase value from that of the tonic response. Figure 14*A* shows the frequency histogram for these values, and Fig. 14*B* shows the same data expressed as a temporal rather than a phase advance. The shapes of the histograms of Fig. 14, *A* and *B*, differ slightly due to different temporal frequencies used to stimulate these cells, because a given phase shift reflects different temporal shifts at different temporal frequencies. Nonetheless, for the stimuli we employed, every cell showed a relative phase and temporal advance for the LT spiking.

DISCUSSION

The major finding of this study is that, in our anesthetized, paralyzed, in vivo preparation, the voltage-dependent LT Ca^{2+} spike can be evoked by visual stimuli in neurons of the cat's lateral geniculate nucleus. We recorded intracellularly at different membrane potentials from 14 geniculate neurons (6 X, 7 Y, and 1 unclassified) and noticed no dramatic difference in LT spiking between the X and Y cells of the study. Every one of these geniculate cells exhibited visually evoked LT spikes. Because of this, each of these cells changed its response mode according to its membrane voltage: at depolarized potentials at which the LT spike was inactivated, each displayed a relay response mode; at more hyperpolarized levels, each entered the burst response mode because of LT spiking, and LT spikes could be reliably activated by visual stimuli. The appearance of LT spiking did, however, seem to alter retinogeniculate transmission, a point discussed in more detail below (see also the following paper, Guido et al. 1992).

Response differences between relay and burst modes

The LT spike seems to provide a boost to assist a hyperpolarized cell in responding to visual stimuli. The boost is evident, because excitatory postsynaptic potentials (EPSPs) that would not be expected to reach firing threshold for action potentials can lead to such firing by evoking an LT spike. Although it is clear that overall responsiveness generally declines with hyperpolarization (e.g., Figs. 7 and 8), the slope of this relationship is shallower than would be expected without the presence of the voltage-dependent Ca^{2+} conductance. Figure 8*A* shows that, in some cases, the LT spike even causes an increase in responsiveness over certain ranges of hyperpolarization. Although the LT spike may amplify retinogeniculate transmission during hyperpolarization of the relay cell, it seems to do so at the expense of

response linearity (see Fig. 11). This is further documented and discussed in the following paper (Guido et al. 1992).

This nonlinear amplification of the LT spike does not seem to affect the cell's spatial properties; that is, spatial tuning of cells is not obviously affected by changed membrane potentials that cause a switch in the mode of firing between the relay and burst modes (Figs. 5 and 6). Although we did not obtain complete temporal tuning functions at different membrane potentials, it seems clear from Fig. 13 that cells faithfully respond during the burst mode in temporal phase with the visual stimulation. Thus, at least during visual stimulation and in vivo recording, we never saw evidence that the burst mode of cells results in rhythmic discharge of LT spikes independent of visual stimulation, and even during spontaneous activity, such rhythmic discharges were not seen (see Fig. 13). We did notice one temporal difference between burst and tonic responses: when they both occurred in response to a visual stimulus, the LT spike and its associated burst of action potentials always preceded the tonic response. It thus seems that the nonlinear response amplification created by the LT spike also speeds up the transmission of this signal to cortex. Although the data from our intracellular recordings regarding spatiotemporal properties of the relay and burst response modes are limited because of our limited cell sample, our extracellular study (Guido et al. 1992) supports these conclusions.

Firing patterns of the relay and burst response modes

As noted in RESULTS, the pattern of action potentials differs significantly between the relay and burst modes of firing. The main difference is interspike interval, which always is ≤ 4 ms during LT spiking. However, when in the relay mode, particularly when strongly excited and depolarized, a geniculate neuron can respond with interspike intervals ≤ 4 ms, which we have called HT bursting. Interspike interval alone is thus an insufficient criterion to distinguish relay from burst firing. We find that the additional criterion of a silent period before the burst does reliably distinguish these response modes. These criteria to identify LT spikes are consistent with the one we have previously described for spontaneous activity (Lo et al. 1991).

The requisite silent period before the burst that identifies the LT spike can be explained on the basis of prior in vitro and in vivo studies, which have shown that the LT spike has a temporal as well as a voltage dependency (Deschênes et al. 1984; Jahnsen and Llinás 1984a,b). To deactivate the underlying Ca^{2+} conductance, it is necessary to hyperpolarize the cell membrane for a period of time. Although the required minimum period of time for hyperpolarization to deactivate has not yet been fully explored as a function of membrane potential, it is clear that very brief hyperpolarizing episodes cannot deactivate the LT spike. Because membrane hyperpolarization is incompatible with action potential generation, it follows that an LT burst can occur only after a certain period of no action potentials, as might be seen during periods of strong hyperpolarization. In contrast, the HT bursting seen during the relay mode occurs only when the cell is quite depolarized (see RESULTS).

One difficulty with studying the effects of LT spiking on

retinogeniculate transmission of visually evoked responses is identifying the relay and burst response modes. This can be done with intracellular recording, but maintaining such recording while measuring receptive field properties at different membrane potentials is difficult and low in yield. However, the establishment of criteria based solely on the pattern of action potential firing means that these different response modes can be reliably distinguished during conventional extracellular recording. This permits more detailed receptive field studies of the effects of the relay and burst response modes from a larger number of neurons, which is precisely what we have done in the following paper (Guido et al. 1992).

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