Visual Response Properties of Burst and Tonic Firing in the Mouse Dorsal Lateral Geniculate Nucleus

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Submitted 29 April 2004; accepted in final form 11 December 2004

Grubb, Matthew S. and Ian D. Thompson. Visual response properties of burst and tonic firing in the mouse dorsal lateral geniculate nucleus. J Neurophysiol 93: 3224–3247, 2005. First published December 15, 2004; doi:10.1152/jn.00445.2004. Thalamic relay cells fire action potentials in two modes: burst and tonic. Previous studies in cats have shown that these two modes are associated with significant differences in the visual information carried by spikes in the dorsal lateral geniculate nucleus (dLGN). Here we describe the visual response properties of burst and tonic firing in the mouse dLGN. Extracellular recordings of activity in single geniculate cells were performed under halothane and nitrous oxide anesthesia in vivo. After confirming that the criteria used to isolate burst spikes from these recordings identify firing events with properties described for burst firing in other species and preparations, we show that burst firing in the mouse dLGN occurs during visual stimulation. We then compare burst and tonic firing across a wide range of visual response characteristics. While the two firing modes do not differ with respect to spatial summation or spatial frequency tuning, they show significant differences in the temporal domain. Burst spikes are phase advanced relative to their tonic counterparts. Burst firing is also more rectified, possesses sharper temporal frequency tuning, and prefers lower temporal frequencies than tonic firing. In addition, contrast-response curves are more step-like for burst responses. Finally, we present analyses that describe the stimulus detection abilities and spike timing reliability of burst and tonic firing.

INTRODUCTION

At its most abstract and fundamental level, the role of the dorsal lateral geniculate nucleus (dLGN) is to feed the cerebral cortex with visual information. Most of this information comes from the retina the afferents of which provide the “driving” influence that determines the large majority of geniculate single cell response properties (e.g., Hubel and Wiesel 1961; Sherman and Guillery 1996, 2002; Usrey et al. 1999). However, retinal afferents do not represent the large majority of inputs to the dLGN. Dominating the dLGN’s afferent population are the 85–90% of synapses that provide “modulating” influences on geniculate processing (e.g., Sherman and Guillery 1996, 2002). Although these modulatory inputs cannot change fundamental receptive field (RF) properties established by retinal drivers, they can produce subtle alterations in the response properties of dLGN neurons, such increasing or decreasing the magnitude of visually evoked responses (e.g., Uhlrich et al. 1995, 2002). Thus due to modulatory inputs onto dLGN cells, the thalamic relay of visual information from retina to cortex becomes a dynamic operation.

One important way in which modulatory inputs to the dLGN can alter the retinocortical transfer of visual information is by changing the firing mode of geniculate relay cells. These neurons, like all thalamic relay cells, can fire action potentials in two different modes: “burst” and “tonic.” As demonstrated by in vitro recordings (e.g., Jahnsen and Llinas 1984; Kim et al. 2001; Ramcharan et al. 2000a; Sherman 2001; Zhan et al. 1999), firing mode is controlled by a T-type calcium channel that produces $I_T$, a voltage- and time-dependent current. $I_T$ is inactivated at relatively depolarized membrane potentials (>60 mV). In this state, depolarizing inputs stimulating a geniculate relay cell will produce conventional, tonic action potentials. If the cell’s membrane potential is relatively hyperpolarized for >100 ms, however, $I_T$ is de-inactivated. Cell stimulation then produces a depolarizing waveform carried predominantly by calcium ions, known as a low-threshold spike (LTS). This LTS is usually accompanied by a burst of sodium-based action potential firing. Modulatory geniculate inputs that produce (long-lasting) depolarizations or hyperpolarizations of cell membrane potentials can therefore switch relay cell responses from burst to tonic firing, and vice versa.

One of the most important situations in which such burst-tonic switches occur is the transition between sleep and wakefulness. Thalamocortical relay neurons in naturally sleeping animals display rhythmic bouts of bursting (McCarley et al. 1983; Weyand et al. 2001), which in vitro studies have established are due to cyclic interactions between $I_T$ and a depolarizing, hyperpolarization-activated current $I_h$ (McCormick and Bal 1997). With the transition from slow-wave sleep to wakefulness, modulatory inputs from the brain stem produce a progressive depolarization of thalamocortical relay cells. This inactivates $I_T$, decreasing burst firing and promoting tonic responses (McCarley et al. 1983; McCormick and Bal 1997).

Because rhythmic burst firing is most prominent during slow-wave sleep (McCarley et al. 1983; Weyand et al. 2001) and because during slow-wave sleep the responsiveness of dLGN neurons to RF stimulation is decreased (Livingstone and Hubel 1981), it might seem that burst firing in thalamocortical relay cells is unimportant as a carrier of sensory information. However, studies in the past decade have begun to uncover the possibility that both burst and tonic firing modes represent specific signals in sensory processing. Lu et al. (1992) used intracellular recordings in vivo to demonstrate that burst responses occur in the cat dLGN during periods of visual stimulation. These recordings also showed that burst spikes could be accurately identified purely on the basis of temporal patterns in spike firing (Lu et al. 1992), allowing less techni-
cally demanding extracellular recordings to confirm that bursting, like tonic firing, occurs during sensory stimulation in anesthetized (e.g., Guido et al. 1992) and awake behaving (e.g., Fanselow et al. 2001; Guido and Weyand 1995; Ramcharan et al. 2000b; Swadlow and Gusev 2001; Weyand et al. 2001) animals. Furthermore, while burst and tonic dLGN spikes carry similar amounts of visual information (Reinagel et al. 1999), recordings in the cat dLGN have shown that the type of visual information transmitted by the two firing modes can be significantly different. Among other differences, burst spikes in the cat follow temporal changes in a sinusoidal stimulus less faithfully (Guido et al. 1992; Lu et al. 1992), offer better stimulus detection (Guido et al. 1995), are more tightly tuned for TF (Mukherjee and Kaplan 1995), and have more reliable timing (Guido and Sherman 1998) than their tonic counterparts. Changes in firing mode in thalamic relay cells might therefore affect the information relayed by those cells to cortex.

Although studies in the cat have identified several differences in the stimulus coding properties of burst and tonic spikes in the dLGN (Guido and Sherman 1998; Guido et al. 1992, 1995; Lu et al. 1992; Mukherjee and Kaplan 1995) (see Discussion), the comprehensive set of data gathered in our laboratory concerning the response properties of mouse dLGN neurons (Grubb and Thompson 2003) offers an opportunity to compare burst and tonic firing across a large range of visual response characteristics. As well as providing a full and detailed description of the functional differences between these two firing modes, such a comparison will also offer an indication as to whether such differences are conserved across mammalian species. We know that the properties of the thalamic $I_F$ current and its accompanying burst activity are similar across different mammals (guinea pig: Jahnzen and Linas 1984; McCormick and Feeser 1990; cat: Zhan et al. 1999; monkey: Ramcharan et al. 2000a; mouse: Kim et al. 2001), but it is unknown whether the distinct visual response characteristics of burst and tonic firing (Guido and Sherman 1998; Guido et al. 1992, 1995; Lu et al. 1992; Mukherjee and Kaplan 1995) are a common feature of mammalian visual processing at thalamic level. The mouse dLGN data presented here represent a first step toward addressing this issue.

Perhaps more importantly, though, studying the visual coding properties of burst and tonic firing in the mouse thalamus opens up the study of firing mode function to genetic manipulation techniques. In mammals, these techniques are most advanced in mice, where they can be invaluable tools for investigating neuronal function at the molecular level. In particular, because the switch between burst and tonic thalamic firing modes can be determined by a single ion channel, investigations of firing mode function might especially benefit from approaches involving genetic manipulation. Mice that lack the T-type calcium channel underlying $I_F$ have been generated (Kim et al. 2001), but before the consequences of this mutation on visual processing at the thalamic or cortical level can be investigated, we need to know the probable contribution of this channel in the normal visual system. We can start to address this issue by investigating the basic visual response properties of burst and tonic firing in the wild-type mouse dLGN. In addition, genetic manipulations might be extremely useful in dissecting the molecular components underlying the control of response mode in thalamic relay cells. Various modulatory systems have been proposed to switch thalamic relay cells from burst to tonic mode or vice versa (e.g., Godwin et al. 1996; Lu et al. 1993; Uhrlrich et al. 2002). Manipulating the receptors or other molecular machinery involved in this modulation could be extremely informative concerning the circuitry that controls or does not control thalamocortical information transmission.

As an essential precursor to such studies, we demonstrate here that burst-like events can be identified in extracellularly recorded responses of mouse dLGN cells and that these events have some properties expected of thalamic burst firing. We go on to show that burst firing in the mouse dLGN occurs during visual stimulation. Finally, we investigate the visual response characteristics of burst and tonic spikes in the mouse dLGN across a broad range of stimulus parameters.

**Methods**

**Animals**

Animals were adult (>3 mo of age) pigmented C57Bl/6J mice of either sex weighing 20–36 g (Harlan Olac, Oxon, UK). Mice were housed under a 12:12-h cycle of light and dark. All procedures were conducted under the auspices of United Kingdom Home Office project and personal licenses held by the authors.

**Electrophysiology**

We carried out electrophysiological recordings as described previously (Grubb and Thompson 2003; Grubb et al. 2003). Mice were first anesthetized with an intraperitoneal injection of 25% fentanyl and fluanisone (Hynorn, Janssen Animal Health, Bucks, UK) and 25% midazolam, Down, UK) administered at 2.7 μg/g. Surgical anesthesia was maintained throughout the setup procedures by subsequent applications of this induction dose. To prevent the accumulation of bronchial secretions, 5 μl atropine sulfate (600 μg/ml, Animalcare, Yorks, UK) was given subcutaneously. A tracheotomy was then performed (Schwarte et al. 2000), and a small plastic tube (1 mm OD, 0.5 mm ID, SIMS Portex, Kent, UK) inserted and secured by ligature around the trachea. Application of a spot of cyanoacrylate glue added extra stability by attaching the tube to the skin of the upper thorax.

The animal was situated in a custom-built head holder and secured using a bite bar and ear bars before the tracheal tube was connected to a respiratory pump (MiniVent 845, Hugo Sachs Elektronik, March-Hugstetten, Germany). Pump rate (usually 120–140 strokes/min) and stroke volume (usually 150–200 μl) were adjusted to maintain the expired carbon dioxide level, monitored with a Capstar-100 CO2 analyzer (CWE, Ardmore, PA), at 2.5–4%. Once on the pump, the mice breathed a 1:3 mixture of oxygen and nitrous oxide, along with 1–1.5% halothane for maintained anesthesia. The mice were not paralyzed at any stage, allowing continued assessment of anesthetic depth via the paw pinch response. Throughout the experiment, heart rate was monitored via an ECG (~5 Hz was normal). Core body temperature, measured with a rectal probe, was maintained at ~37°C by combining a high ambient temperature with heat from a thermally-coupled blanket (NP 50-7061-R, Harvard Apparatus, Kent, UK).

A circular craniotomy ~0.5 mm in diameter was made unilaterally above the presumptive location of the dLGN, ~2.5 mm posterior and 2 mm lateral of the Bregma suture (Paxinos and Franklin 2001). A small durotomy was made in the center of this exposed zone and, after any whiskers occluding the eyes had been trimmed, a tungsten-in-glass recording electrode with a 5- to 10-μm exposed tip (Alan Ainsworth, Northants, UK) was lowered vertically into the brain using a microdrive. Changes in background activity while advancing through the brain were used to locate the electrode relative to the
dLGN (Grubb and Thompson 2003). Once in the dLGN, the electrode was advanced or retracted in 5-μm steps. Signals were amplified locally by a FET headstage, further amplified by a Neurolog preamp (NL104; typically 10–20 kΩ), then filtered (Neurolog NL125, low-frequency ~150 Hz, high-frequency ~3,000 Hz) for display on an oscilloscope. A Schmidt trigger was used to isolate responses of single neurons by adjusting the signal threshold; the trigger was also connected to a storage oscilloscope that enabled us to monitor spike shapes and to ensure that no spikes were seen in the refractory period. To isolate the responses of single units, electrode position was adjusted until spikes were deemed to have the same shape, amplitude, and rise time. Responses of optic tract fibers—typically large and fast, with high levels of spontaneous firing and a lack of burst activity—were easily distinguished from cell soma responses. Spike events were then time-stamped with a resolution of 100 μs and were linked to the timing of visual stimulus changes using in-house software written by D. Smyth.

Although we did not apply contact lenses, optical quality in our setup was good and did not degrade over the course of the experiments. As described previously (Grubb and Thompson 2003), no significant correlations were found between time from experiment start (≤13 h) and any measures of spatial tuning in dLGN neurons. Furthermore, within animals, there were no significant differences in these measures between cells recorded in the first versus the second half of experiments (paired t-test or Wilcoxon paired test, depending on normality, P > 0.05). Eye movements under halothane anesthesia were rare and small (Grubb and Thompson 2003; cf. Dräger 1975; Gordon and Stryker 1996; Wager et al. 1980). As described previously (Grubb and Thompson 2003), remapping of RFs 0.5–1 h after their initial localization revealed a mean shift (±SE) in position of only 3.6 ± 0.5° (n = 11). In addition, over a time scale of ~10 min, response features such as phase-locked raster plots (Fig. 5), sinusoidal dependence of cell responses on stimulus phase (Fig. 6), and regular shifts in response phase with increasing temporal frequency (Fig. 5), all suggest that RF position changed very little. Moreover, in a study where properties of burst and tonic firing were always compared within individual tuning curve experiments, small eye movements were unlikely to affect the present findings.

**Stimulus presentation**

Visually responsive neurons were first identified using a hand-held ophthalmoscope. Monochrome visual stimuli were then presented on a cathode ray tube monitor under the control of a Visual Stimulus Generator 2/4 graphics card (Cambridge Research Systems, Cambridge, UK); this had a pseudo-15-bit analog output allowing precise control of the luminance of each pixel on the display and correction for expansive luminance nonlinearities. Stimuli could be presented with 256 linearly spaced gray levels. The display comprised 800 × 600 pixels and at viewing distances between 135 and 400 mm subtended 53–111 × 41–95°. With maximum and minimum luminances of 99 and 2cd/m², respectively, the display was the main source of illumination in an otherwise dim room. The frame rate of the monitor was 160 Hz.

The display was initially placed with a given cell’s presumptive RF at its center; mapping with reverse correlation (Grubb and Thompson 2003) then confirmed this location, or directed movement of the monitor in order that the display covered the entire RF area. Only cells the RFs of which could be localized in this way were subjected to subsequent analysis of response characteristics. Stimuli were presented with both eyes open, but responses in all cells were most likely driven entirely by the contralateral eye: not only are all wild-type mouse dLGN cells monocularly driven (Grubb et al. 2003), but in these investigations, the display was often placed in positions that could be viewed only by the contralateral eye. Furthermore, although it is still possible that interactions between the two eyes could modulate the responses of single mouse dLGN neurons, such interactions are unlikely to affect present analyses in which burst and tonic response properties were compared within cells.

For quantitative investigation of the visual response properties of burst and tonic firing, sinusoidal monochromatic vertical gratings were presented covering the full extent of the display. These gratings were drifting in all experiments except the null test, in which they were stationary and sinusoidally contrast-modulated. In all experiments, all grating parameters were kept constant except the one under study; gratings varying in this parameter were presented in a pseudo-random sequence. Grating parameters in spatial frequency (SF) and temporal frequency (TF) tuning assessments were varied systematically and logarithmically to produce 8 stimuli ranging between ~0.01 and ~1 c/°² inclusive for SF assessments, and between 0.5 and 16 Hz inclusive for TF assessments. In contrast and null phase test assessments, grating parameters were varied systematically and linearly to produce 12 stimuli ranging between 1 and 100% inclusive for contrast assessments and between ~180 and +150° inclusive for null phase tests. Every stimulus repeat also contained a blank stimulus at mean luminance from which spontaneous activity levels were calculated. Each presentation of a grating stimulus in a given repeat occurred for seven cycles; of these only responses from the last five cycles were used to discard any flash responses to the initial presentation of the stimulus. The exception was assessing TF tuning. In these experiments, stimuli ~1 Hz were presented for seven cycles (with the 1st 2 discarded) as usual. However, to ensure capture of enough responses to high TF stimuli, stimuli ~1 Hz were presented for seven seconds, with the first 2 s being discarded to allow for flash responses. At least two repeats of each 7-cycle (or 7 s) stimulus were presented to every cell, although in the majority of cases four repeats were utilized.

**Histology**

Each experiment was ended with an intraperitoneal injection of 0.3 ml pentobarbitone sodium (Sagatal, Rhône Mérieux, Essex, UK). The mouse was then transcardially perfused with phosphate-buffered saline (Sigma, Dorset, UK) followed by 4% paraformaldehyde (TAAB Laboratories, Berks, UK) in 0.1 M phosphate buffer. The brain was then removed, sunk in 30% sucrose for cryoprotection, and sectioned coronally at 30–50 μm on a freezing microtome. Sections were then mounted from distilled water onto gelatinized slides. Subsequent staining for cell bodies with cresyl violet allowed the identification of small (6 μA for 6 s) electrotyc lesions made at precise locations on successful penetrations. Locating these lesions made it possible to reconstruct electrode tracks and localize recorded units. Only cells unequivocally located within the dLGN were included in subsequent physiological analyses.

**Data analysis**

IDENTIFYING BURST AND TONIC RESPONSE COMPONENTS. Spikes forming part of bursts triggered by low-threshold calcium spikes (LTS bursts) were identified from temporal patterns in the firing of dLGN cells. From intracellular recordings in the cat dLGN, Lu et al. (1992) developed criteria that could reliably identify bursts driven by LTS activity: we applied the same criteria here. Specifically, the first action potential in a burst had a preceding silent period of ~100 ms and a following interspike interval (ISI) of ~4 ms. Any subsequent spikes with preceding ISIs of ~4 ms were designated as additional action potentials in a burst. All spikes that did not form part of LTS bursts were designated as part of tonic firing.

RHYTHMICITY. Rhythmicity in spontaneous bursting was assessed after Weyand et al. (2001). For each 5-s presentation of a 51 cd/m² blank screen during which at least four bursts were fired, we calculated the SD of interburst intervals, the latter being defined as the time between the first spikes of consecutive bursts. This SD is a direct measure of burst rhythmicity—perfect rhythmic bursting should...
duce a SD of 0. To assess the statistical significance of each SD measure, we employed a Monte Carlo approach (Weyand et al. 2001). For each blank stimulus presentation, 999 simulations were run in which an identical number of bursts were randomly distributed within the 5-s interval. Standard deviations of interburst intervals were calculated for each simulation and were ranked alongside the original, measured value. The rank of the value of the observed SD then gave a statistical measure of burst rhythmicity: significant rhythmicity ($P < 0.05$) was detected if the observed value fell within the 50 lowest simulated values.

FOURIER ANALYSIS. Fourier analysis was applied separately to a given cell’s tonic and burst spikes. Burst or tonic responses to a given grating stimulus were first binned according to their poststimulus time. One hundred twenty eight bins were used per stimulus cycle, producing a poststimulus time histogram (PSTH) for each stimulus. A fast Fourier transform of these data then converted these PSTH data into amplitudes (in spikes/s) and phases (in degrees or radians) of the harmonics of the stimulus’ modulation frequency (Hochstein and Shapley 1976).

RESPONSE LINEARITY. Linearity of spatial summation was assessed using a linearity measure first applied by Hochstein and Shapley (1976). Over the many stimulus phases presented in a single modified null test, the mean second harmonic (F2) response amplitude was divided by the maximum fundamental harmonic (F1) response amplitude. Linearity values <1 are indicative of linear spatial summation, while linearity values greater than one are indicative of nonlinear spatial summation.

Another measure of response linearity, “rectification,” was calculated over a given tuning curve as follows

$$\text{rectification} = \frac{\sum F_2}{\sum F_2 + \sum F_1},$$

where F2 and F1 are the amplitudes of the second and first harmonics, respectively, in responses fired to a stimulus s. Although rectification might be best measured by comparing the amplitude of F1 response components with the combined amplitudes of all higher response harmonics (F2, F3, F4, and so on), this particular measure was employed here because of its previous use as an indicator of response nonlinearity in cat dLGN cells (Guido et al. 1992; Lu et al. 1992).

CURVE FITTING. All curve fits were carried out using a least-squares minimization algorithm (Grubb and Thompson 2003).

Plots of stimulus SF versus cell F1 response amplitude were fitted with a difference of Gaussians curve (Grubb and Thompson 2003; Rodieck 1965; So and Shapley 1981)

$$R(s) = b + (k_1 - b) \cdot e^{-(s - r_1)^2} - c \cdot e^{-(s - r_1)^2}$$

where $R$ is F1 response amplitude, $s$ is SF, $b$ is baseline response, $k_1$ is the area under the RF center’s Gaussian function, $r_1$ is the relative area under the RF surround’s Gaussian function, and $r_1$ and $r_2$ are the radii of the center and surround Gaussian functions, respectively, at the point each mechanism has reached 1/e of its peak.

Plots of stimulus TF versus cell F1 response amplitude were fitted with an atheoretical function comprising two half-Gaussians (Grubb and Thompson 2003)

$$R(\omega) = b_1 + (a - b_1) \cdot e^{-(\omega - \omega_0)^2} \quad \text{for} \quad \omega < p$$

$$R(\omega) = b_2 + (a - b_2) \cdot e^{-(\omega - \omega_0)^2} \quad \text{for} \quad \omega > p$$

where $R$ is F1 response amplitude, $\omega$ is TF, $p$ is peak TF, $a$ is the response amplitude at optimum TF, $s$ is the Gaussian spread, $b_1$ is the baseline on the low-frequency side of the curve, and $b_2$ is the baseline on the high-frequency side of the curve. Plots of stimulus TF versus cell F1 response phase were simply fitted with a straight line.

Plots of stimulus Michelson contrast versus cell F1 response amplitude were fitted using a hyperbolic function (Albrecht and Hamilton 1982; Grubb and Thompson 2003) of the form

$$R(c) = b + (R_{\text{max}} - b) \cdot \frac{c^a}{c^a + b^a}$$

where $R$ is F1 response amplitude, $c$ is contrast, $R_{\text{max}}$ is maximum response, $b$ is the contrast at which $R$ reaches half of $R_{\text{max}}$, and $a$ is rate of change.

SIGNAL DETECTION ANALYSIS. Receiver operating characteristic (ROC) curves were derived from probability distributions of spike counts obtained during a sampling episode $\tau$ of visually driven ($\tau_v$) and spontaneous ($\tau_s$) activity (Guido et al. 1995). $\tau_s$ comprised half the temporal period of the stimulus and was centered on the phase of the F1 response component. $\tau_v$ comprised an equal period during the presentation of a blank screen (51 cd/m²). For all spikes fired to a given stimulus, a criterion domain was set corresponding to the full range of spike counts (including 0) occurring for $\tau_v$ and $\tau_s$. ROC curves were then obtained by plotting, for each criterion level, the probability $P$(false alarm) of obtaining a criterion response during $\tau_v$ versus the probability $P$(hit) of obtaining a response during $\tau_v$. To produce a nonparametric measure of discrimination performance (Green and Swets 1966; Macmillan and Creelman 1991), the area underneath each ROC curve was then calculated using simple geometry.

MEASURES OF SPIKE TIMING RELIABILITY. Spike timing reliability was assessed using two measures. The first, “reliability,” was applied to all burst or tonic spikes fired to a given stimulus, and represented the mean deviation of spike times from their F1 phase, normalized by the stimulus period

$$\text{reliability} = \frac{\sum |t_i - t_{iF1}|}{p}$$

where $s$ is the time of a given spike $i$, $t_{iF1}$ is the time of the F1 phase, $n$ is the number of spikes, and $p$ is the stimulus period.

First-spike reliability was applied only to the first burst or tonic spikes fired during a particular stimulus cycle and was calculated as the SD of first-spike latencies. Latencies were taken from the start of a half-cycle period centered on the F1 component of either burst or tonic firing, as appropriate

$$\text{first spike reliability} = \frac{\sum \left( f_i - t_0 \right) - \left( \sum \left( f_i - t_0 \right) / n \right)}{n}$$

where $f$ is the time of a given first spike $i$, $t_0$ is the start time of the half-cycle period, and $n$ is the number of first spikes. This measure was only calculated when $n \geq 10$.

Statistical analysis

Sample sizes in this study were large enough ($n > 30$) to reliably assess their Normality using a Kolmogorov-Smirnov test. Those deemed Normal were described using the means ± SE and were compared using parametric tests. Those deemed non-normal were described using the median and were compared using nonparametric tests. Paired tests were used to compare burst and tonic spikes fired by the same cell to the same stimuli. All comparison tests were two-tailed, with the level of significance set at 0.05.

RESULTS

Identifying burst firing in the responses of mouse dLGN cells

Visual response properties of burst and tonic firing were assessed in 102 mouse dLGN cells, all with classic geniculate
on- or off-center receptive fields (see Grubb and Thompson 2003). Responses were recorded from cells with RFs at visual eccentricities ranging from 0 to 92° (means ± SE: 44 ± 3°). Because the response properties of mouse dLGN cells change very little with increasing visual eccentricity (Grubb and Thompson 2003), we did not limit our analysis to cells with RFs in particular regions of visual space.

Burst firing in the responses of cells to sinusoidal grating stimuli was identified on the basis of temporal patterns of spikes. The first spikes in bursts were preceded by ≥100 ms of silence and were followed by another spike within 4 ms. Subsequent spikes in bursts followed at ISIs of ≤4 ms (Lu et al. 1992) (see METHODS). Figure 1 illustrates the way in which the responses of mouse dLGN cells to drifting sinusoidal grating stimuli were analyzed using a double interspike interval (ISI) plot, revealing distinct temporal patterns of firing associated with burst responses. For each spike, subsequent ISI is plotted against preceding ISI. The distribution of points de-

FIG. 1. Separating burst and tonic components in the responses of mouse dorsal lateral geniculate nucleus (dLGN) cells. Each interspike interval (ISI) plot shows spikes fired during a particular tuning curve experiment. Each spike, represented by a single dot, is plotted according to the period between it and the spike preceding it (ISI pre, x axis) and the period between it and the spike following it (ISI post, y axis). Note the logarithmic axes. In the “all spikes” plot (left), spikes are clustered into prominent groups. In box 1, spikes are preceded by ≥100 ms of silence and are followed by another spike within 4 ms. These spikes are the first spikes of bursts. In box 2, spikes possess preceding and preceding ISIs of no more than 4 ms. Most of these spikes are the middle spikes of bursts. In box 3, spikes have preceding ISIs of no more than 4 ms and are followed by variable periods of inactivity. Most of these spikes are the last spikes of bursts. Spikes falling within other areas of the plot are all included in tonic firing. The line marked TF displays the temporal period of the stimulus. The “burst spikes” plot (center) shows just those spikes that are part of bursts. The “tonic spikes” plot (right) shows all spikes that are not part of bursts. A: a cell dominated by burst firing. Most spikes fall within boxes 1–3. Note that the x-axis position of spikes in box 1 and the y-axis position of spikes in box 3 are largely determined by the period of the stimulus. The y-axis position of cluster 1 and the x-axis position of cluster 3, however, are determined by features of thalamic burst firing. B: a cell with prominent burst and tonic firing. This cell’s spikes show temporal patterns in addition to the clusters in boxes 1–3. Region 4 outlines last spikes in bursts that are followed by a relatively long spike-free period. These burst spikes are probably the last stimulus-driven spikes fired in a given stimulus cycle. In contrast, region 5 outlines last spikes in bursts that are quickly followed by tonic firing. Region 6, where tonic spikes are followed by a long dead time almost equal to the stimulus period, outlines last spikes in stimulus cycles that did not evoke any burst responses. C: a cell dominated by tonic firing. Box 2 is empty and only very few 2-spike bursts occurred.
pends on the temporal properties of the stimulus and, more importantly, the balance between burst and tonic firing. The significance of the latter can be seen by comparing Fig. 1, A and C, in which example cells fire almost all of their spikes as part of burst or tonic firing, respectively. Figure 1B illustrates the more common occurrence in which both burst and tonic spikes contribute to a cell’s responses.

In the double ISI plots of Fig. 1, distinct clusters of spike ISI distributions can be seen. Some of the outlined zones are constrained by the features of burst firing; others are determined by the temporal structure of the visual stimulus. Zones 1–3 are all conditioned by the properties of bursts. Zone 1 contains the first spikes in bursts; these spikes are preceded by \( \geq 100 \text{ ms} \) of silence and are followed by another spike within 4 ms. Spikes within bursts, with pre- and post-ISIs of \( \leq 4 \text{ ms} \), form the majority of spikes in zone 2, while the \( x \)-axis position of the cluster in zone 3 is due to these spikes being mostly the last spikes in bursts—they follow within 4 ms of a previous spike but are themselves followed by \( > 4 \text{ ms} \) of nonspiking “dead” time. In the example cell in Fig. 1A, dominated by burst responses, these zones contain almost all of the spikes fired. However, the temporal structure of the stimulus also affects the positioning of clusters in double ISI plots: assuming a neuron fires spikes to every stimulus cycle, maximum pre- and post-ISIs are constrained by the grating’s temporal frequency (TF). In the example cell in Fig. 1C, dominated by tonic responses, the majority of spikes fall outside zones 1–3 but are constrained by the stimulus’ TF. The example cell in Fig. 1B, in contrast, displays a complex ISI pattern indicative of important contributions from both burst and tonic firing. This produces a broad distribution of post-ISIs in zone 3: while many of these last spikes in bursts are the last fired in a cycle and are thus constrained largely by stimulus TF (zone 4), many others are followed by tonic firing and thus possess much shorter post-ISIs (zone 5). Similarly, within spikes fired as part of tonic firing, spikes clustered in zone 6 are the last spikes fired in a cycle, and those clustered in zone 7 are the first fired in a cycle.

Firing in mouse dLGN cells thus conforms to particular temporal patterns, some of which correspond well to those predicted by the properties of intracellularly recorded LTS bursts in the cat dLGN (Lu et al. 1992). Separating mouse dLGN cell responses into burst and tonic components on the basis of the preceding criteria is therefore nonarbitrary: it is effective in classifying preexisting temporal patterns of neuronal firing. However, this classification is not quite perfect. Figure 1 shows that “tonic” spikes can sometimes occupy ISI clusters (zones 2 and 3) dominated by burst firing. Indeed, Lu et al. (1992) concede that their criteria are conservative, allocating to tonic firing \(~5–10\%\) of spikes that actually form part of bursts.

**Characteristics of burst firing in the mouse dLGN**

Bursting appears to be a well-conserved thalamic phenomenon. As assessed in vitro, the properties of the \( I_T \) current and its accompanying burst activity are similar across thalamic nuclei (Jahnsen and Llinas 1984; Ramcharan et al. 2000a) and across different mammalian species (guinea pig: Jahnsen and Llinas 1984; McCormick and Feeser 1990; cat: Zhan et al. 1999; monkey: Ramcharan et al. 2000a; mouse: Kim et al. 2001). Furthermore, it was shown in the previous section that distinct patterns of firing in the mouse dLGN can be identified by criteria used to identify burst spikes in other species (cat: Lu et al. 1992; monkey: Ramcharan et al. 2000b). However, we wanted to be more confident that the bursting criteria used here were identifying the same LTS-based phenomenon studied in other species. We therefore investigated whether certain features of burst firing reported to occur in those species also existed in the mouse dLGN.

**Bursts are longer if preceded by greater periods of silence**

It is known from in vitro recordings in the cat (Zhan et al. 1999) and monkey (Ramcharan et al. 2000a) that the number of spikes in a burst is not related to the strength of the depolarizing input stimulus but rather to the hyperpolarization of the membrane potential when that depolarizing stimulus arrives. In vivo, membrane hyperpolarization should depend on the degree of inhibition on a cell. Such inhibition will vary over time, but might have most chance to hyperpolarize a cell when that cell has been silent for an extended period. One might therefore predict that, although nonspiking “dead” time and membrane hyperpolarization need not go hand in hand, the number of spikes in bursts in vivo might be greater when bursts are preceded by longer periods of silence. In fact, across all bursts recorded in all experiments in all cells here, a small but significant positive relationship was noted between burst length and the duration of the preburst silent period (Spearman \( r = 0.16, P < 0.0001, n = 42,400 \)). These data were summarized by taking the median preburst silent period for different burst lengths (Fig. 2A). For bursts consisting of \(<5\) spikes, which represented \( >99\% \) of all bursts recorded, there is a clear increase in the median period of preburst silence with increasing burst length. For larger bursts, this relationship breaks down. This could be due to the relatively small sample of long bursts. Alternatively, Rowe and Fischer (2001) used paired retinal and geniculate recordings to show that the first spikes in thalamic bursts are usually stereotypical events triggered by a single retinal spike but that later spikes in long bursts can be triggered directly by subsequent retinal action potentials. In other words, long bursts in vivo may not be long because of a large initial membrane hyperpolarization (Zhan et al. 1999), but because they are triggered by two or more retinal action potentials closely spaced in time. If the latter is true, the relationship between preburst dead time and burst length might be expected to break down for the very longest bursts recorded.

**Longer bursts have shorter initial intraburst ISIs**

It has been previously reported that burst length in the cat (McCarley et al. 1983) and human (Radhakrishnan et al. 1999) thalamus is correlated with the duration of the first within-burst ISI. This was confirmed for the mouse dLGN here. Across all bursts there was a strong negative relationship between the first ISI within a burst and burst length (Spearman \( r = -0.41, P < 0.0001, n = 42,400 \)). Taking median initial intraburst ISI values for each burst length also reveals that bursts are longer when the first intraburst ISI is shorter (Fig. 2B).

**ISI duration increases within bursts**

Another widely reported feature of thalamic bursting is that ISIs become progressively longer within bursts (e.g., McCarley...
et al. 1983; Radhakrishnan et al. 1999; Reinagel et al. 1999). This feature was also observed here. Grouping data across bursts in the mouse dLGN, there was a significant positive correlation between within-burst ISI number and ISI duration (Spearman $r = 0.22, P < 0.0001, n = 77,384$). Taking median ISI duration values for each ISI position also shows a gradual increase in duration from the average first through to the average fourth ISI in a burst (Fig. 2C1). Later within-burst ISIs do not continue the trend but represent $<1\%$ of the ISIs analyzed and, as noted in the preceding text, might be expected to be less stereotyped than earlier within-burst ISIs (Rowe and Fischer 2001). A more powerful demonstration of increasing ISI duration within bursts comes, however, from analyzing differences in within-burst ISIs within individual bursts. Figure 2C2 shows that the median difference in duration between a within-burst ISI and its preceding within-burst ISI is always positive. All median difference values here are significantly greater than zero (Wilcoxon rank sum test, $P < 0.05$). In other words, any given intraburst ISI tends to be shorter than the intraburst ISI that follows it.

**Bursts are stereotyped events**

Following Reinagel et al. (1999), the coefficient of variation (CV) of burst length was calculated for each experiment. Defined as the SD divided by the mean of any given sample, the CV should be $\sim 1$ if sample values vary by chance. Here, most bursts (47%) consist of only two spikes. This stereotyped length is reflected in low CV values in every tuning curve experiment (Fig. 2D). The median CV value across all tuning curve experiments was 0.25, strikingly similar to mean values reported for cat X (0.25) or Y (0.23) cells (Reinagel et al. 1999).

**Rhythmicity of spontaneous burst firing**

Spontaneous bursting in the dLGN neurons of naturally sleeping animals often occurs rhythmically (McCarley et al.
1983; Weyand et al. 2001), with rhythmicity established by cyclic interactions between two depolarizing geniculate currents \( I_T \) and \( I_h \) (McCormick and Bal 1997). We assessed whether spontaneous bursting is also rhythmic in the mouse dLGN under conditions of halothane anesthesia. Our analysis was based on 5-s periods of spontaneous activity in which the stimulus display consisted of a blank screen at mean luminance (51 cd/m\(^2\)); such blank presentations occurred once for every repeat of sinusoidal grating stimuli in a given tuning experiment (see METHODS). Rhythmicity was assessed by analyzing interburst intervals during such 5-s spontaneous epochs. For each epoch that contained at least four bursts, we calculated the SD of interburst intervals, with this SD measure being directly related to bursting rhythmicity: perfect rhythmic bursting would produce a SD of zero. We then assessed the statistical significance of each SD rhythmicity measure using Monte Carlo simulations that ranked the observed rhythmicity value among 999 simulated values (see METHODS).

Examples of burst timing in the responses of a single mouse dLGN cell during several 5-s spontaneous epochs are presented in Fig. 3A. These plots show that evenly-spaced bursts produce significant rhythmicity values and also demonstrate that spontaneous bursting in the mouse dLGN could alternate between rhythmic and nonrhythmic behavior over time in the same neuron. The histogram in Fig. 3B presents \( P \) values for all 234 spontaneous epochs (involving 45 cells) that contained at least four bursts. Forty four of these epochs (19\%, involving 22 cells) were accompanied by significantly rhythmic bursting \((P < 0.05)\). Of the 22 cells showing rhythmic spontaneous bursting, most \((N = 12)\) did so in only a single epoch, although 3 cells had five rhythmic bursting epochs each. Rhythmicity in spontaneous bursting therefore occurs \(~20\%\) of the time in the dLGN cells of a halothane-anesthetized mouse and is restricted to around half of geniculate neurons under such conditions, although a minority of neurons display rhythmic bursting rather often. This level of rhythmicity is considerably lower than the \(~47\%\) of rhythmic episodes observed in the naturally sleeping cat (Weyand et al. 2001). The discrepancy could arise because our spontaneous epochs were 5 s long, whereas Weyand et al. based their analysis on “burst bouts” of \(~2\) s. However, when we also analyzed the rhythmicity of such 2-s burst bouts in our mouse preparation, we observed a reduction in rhythmic episodes: 48 of 438 bouts (11\%) displayed significantly rhythmic firing. dLGN cells in anesthetized mice therefore do burst rhythmically but compared with geniculate neurons in naturally sleeping animals do so only rather rarely.

**Burst firing is produced by visual stimulation**

Neurons in the mouse dLGN fire spikes that can be identified as part of bursts (Fig. 1) and that can be shown to possess features characteristic of burst firing in other species and preparations (Fig. 2). But do these burst spikes encode visual information? A first, crude step toward answering this question comes from a comparison of bursting prevalence during periods of spontaneous and visually driven activity. Every investigation of a dLGN cell’s responses to a certain stimulus parameter (known as a “tuning experiment”) involved presenting a pseudorandom series of sinusoidal grating stimuli which varied in the parameter under study (spatial frequency, contrast, etc.; see METHODS) and also included the presentation of a blank stimulus at mean luminance. For each such tuning experiment, we calculated the percentage of spikes forming part of bursts (burst \%) over all presentations of all grating stimuli (visually driven activity), and over all periods of blank screen presentation (spontaneous activity). We recognize that this approach does not compare matched periods of visually driven and spontaneous spiking, but our use of a percentage measure of bursting prevalence should compensate for this.

Although some cells’ visual firing was dominated by burst firing (maximum burst \% = 78\%; see Fig. 1A), in most experiments bursts occupied a small fraction of all spikes fired...
(Fig. 4A1). However, burst percentage values for periods of spontaneous activity were usually even lower with many cells not firing any burst spikes in the absence of visual stimulation (Fig. 4A2). Indeed, across all tuning experiments (n = 323 in 102 cells), burst percentage values were significantly higher for visually driven versus spontaneous activity (visual median: 19.8%; spontaneous median: 11.1%; Wilcoxon matched pairs test, P = 0.002; Fig. 4B). Because this analysis was applied across all visual stimuli in a given experiment, not just those producing the highest firing rates, this is a rather clear demonstration that bursting is more prevalent when cells are responding to visual stimuli. This begs the question: exactly what visual information does burst firing carry?

**Burst spikes are fired in response to earlier stimulus phases**

Investigations into the relative timing of burst and tonic spikes during presentation of sinusoidal gratings suggest that burst spikes might carry important information about stimulus onset. Figure 5A shows the burst and tonic responses of a mouse dLGN cell to a sinusoidal grating of optimum spatial and temporal frequencies. As often reported by others (e.g., Guido et al. 1992, 1995; Lu et al. 1992) burst spikes, when they occur, tend to occur at the start of a response to a given stimulus cycle. Tonic spikes tend to occur a little later. This precedence of burst firing can have two possible mechanisms: burst spikes can have shorter latencies than tonic spikes or they can be triggered by earlier phases of the stimulus. The relative contribution of each of these candidate mechanisms can be assessed using plots of stimulus temporal frequency (TF) versus cell F1 response phase. The slope of a line relating these two variables can be used as a measure of response latency (Grubb and Thompson 2003; Hawken et al. 1996). In addition, the y intercept of the line can give an indication of relative response phase (e.g., Guido et al. 1992; Mukherjee and Kaplan 1995). In mouse dLGN cells, burst responses to low TFs are usually phase advanced relative to tonic responses to the same stimuli. At high TFs, though, response phases for the two firing modes tend to be similar (Fig. 5B). This makes the TF-F1 phase line steeper for burst firing, meaning that burst spikes possess longer latencies than tonic spikes. Across all TF experiments, burst latencies were significantly longer than the latencies of tonic spikes fired to the same stimuli (burst median: 106 ms; tonic: 90 ms; Wilcoxon matched pairs test, P = 0.001; Fig. 5C). However, burst spikes still occur earlier than tonic spikes because they are triggered by an earlier phase of the stimulus. Phase advances for burst spikes, taken by subtracting tonic from burst y-intercept values in each experiment, were almost all positive, with the sample median being significantly different from zero (more positive values reflect earlier phases; 1-sample t-test, P < 0.0001; Fig. 5D). Despite their longer latency, burst spikes usually carry the first stimulus information relayed to cortex.

**Response linearity**

When all spikes are taken into consideration, almost all mouse dLGN cells sum inputs in a linear manner across their RFs (Grubb and Thompson 2003). It is unlikely that such linear behavior could have a substantially nonlinear component, so it was reassuring to note that in almost all mouse dLGN cells both burst and tonic spikes show linear spatial summation. The plots in Fig. 6A display typical results of modified null tests (Hochstein and Shapley 1976) applied separately to the burst and tonic components of cells’ responses. These tests involve displaying stationary sinusoidal gratings the contrast of which is sinusoidally modulated in time, at various spatial phases across a cell’s RF. Null tests were always initially applied with stimuli at a cell’s optimal spatial frequency (SF) but were often repeated at higher SFs because nonlinearities of spatial summation can become more prevalent as SF increases (e.g., Hochstein and Shapley 1976). Twenty-eight cells were tested at optimal SF, 12 at twice optimal SF, and 3 at three times optimal SF. Temporal frequency of these stimuli was 1 Hz (11 cells) or optimal (17 cells), maximum stimulus contrast was
70%. By convention, fundamental (F1) response amplitudes are represented as negative in null test plots when their phase differs by 90–270° from that of the maximum response; this explains why burst and tonic plots from the same cell sometimes look like mirror images of each other.

In cells that sum inputs linearly across their RFs, responses to counterphased stimuli should be largest at the fundamental (F1) stimulus harmonic and the amplitude of this harmonic should vary sinusoidally with stimulus phase (Hochstein and Shapley 1976). In addition, a linearly summating cell should possess two null phases where excitatory and inhibitory inputs vary sinusoidally with stimulus phase (Hochstein and Shapley 1976). In almost all cases in our mouse dLGN sample (27/28 cells, 42/43 null tests, in a null test plot (Hochstein and Shapley 1976). In almost all son 1966). These null phases are represented by zero crossings are perfectly balanced across the RF (Enroth-Cugell and Rob-
high maximum F1 response in its burst firing meant that only its tonic component possessed a linearity value greater than unity, at 1.64.

Across all null test experiments, linearity values of burst spikes were not significantly different from those of tonic spikes fired to the same stimuli (burst means \( \pm \) SE: 0.42 \( \pm \) 0.02; tonic: 0.37 \( \pm \) 0.04; paired \( t \)-test, \( P = 0.17 \); Fig. 6B). Both burst firing and tonic firing in the responses of mouse dLGN cells therefore sum spatial inputs in an equally linear manner. Although burst and tonic firing do not differ in terms of their linearity of spatial summation, they do differ on a very different measure of response linearity, rectification. The response of a linear neuron to a sinusoidal grating stimulus should be sinusoidal, with a dominant F1 component (e.g., Shapley and Lennie 1985). However, even when a neuron displays wholly linear spatial summation its response to a sinusoid may not be sinusoidal because of response rectification: when spontaneous activity is low and visual responses strong and transient, cells cannot decrease their responses to the nonpreferred half cycle of a given grating, and may not precisely follow the temporal form of the grating’s preferred half cycle. Such response rectification acts to increase all nonfundamental harmonics in a
cell’s responses. Because burst spikes tend to be high-frequency and transient, one might expect their rectification to be greater. Indeed, over all tuning experiments ($n = 332$ in 102 cells), the “rectification” of burst spikes (Eq. 1) was significantly greater than that of tonic spikes fired to the same stimuli (burst median: 0.46; tonic: 0.36; Wilcoxon matched pairs test, $P < 0.0001$; Fig. 6C). Thus although both burst and tonic spikes display linear spatial summation, burst spikes are significantly more nonlinear in terms of response rectification.

Spatial frequency tuning
As well as being identical with regard to spatial summation, burst and tonic spikes in the mouse dLGN are no different with regard to their SF tuning properties. SF tuning was assessed by fitting separate Difference of Gaussians (DoG) curves (Eq. 2) to the F1 amplitudes of burst and tonic responses to sinusoidal gratings of various SFs. Temporal frequency of these stimuli was 1 Hz, contrast was 70%. The DoG equation (Rodieck 1965, So and Shapley 1981) models the center and surround mechanisms of a cell as symmetrical, antagonistic Gaussian functions, and can provide measures of the strengths and sizes of a given cell’s center and surround RF regions. Because a DoG curve also provides a smooth fit to raw data, it can also be used to precisely calculate a cell’s SF optimum (or peak) and cutoff (taken as the high SF at which response amplitude decayed to 1% of its maximum). However, due to the large size of RFs in the mouse dLGN, coupled with the limited size of our stimulus display at appropriate viewing distances, we were unable to accurately describe a cell’s full low-SF roll-off (see Grubb and Thompson 2003).

As shown in Fig. 7A, DoG curves fitted to burst and tonic

![Fig. 7. Spatial frequency tuning of burst and tonic spikes in the mouse dLGN. A: examples of burst and tonic spatial frequency (SF) tuning curves in 3 neurons. • and ○ show F1 response amplitudes to drifting sinusoidal gratings of various SFs; —, the best fit to these data of a Difference of Gaussians model (see METHODS). Note the different y-axis scales for the 3 examples, and the logarithmic x axis. Across these examples, and the mouse dLGN sample as a whole, the shape of SF tuning curves tended to be very similar for burst and tonic components of a given cell’s responses. In some cells, burst responses were stronger than tonic responses (A1). More commonly, tonic firing was stronger than burst firing (A2). In other cells, burst and tonic tuning curves were similar in amplitude as well as shape (A3). Spatial tuning parameters for all 3 cells: cell 1: burst firing: $k_c$ 18.4, $k_s$ 0.67, $r_c$ 2.8°, $r_s$ 4.0°, peak 0.063 c/°, cutoff 0.27 c/°; tonic firing: $k_c$ 6.7, $k_s$ 0.70, $r_c$ 2.9°, $r_s$ 6.0°, peak 0.064 c/°, cutoff 0.25 c/°; cell 2: burst firing: $k_c$ 7.4, $k_s$ 0.69, $r_c$ 3.8°, $r_s$ 17.0°, peak 0.031 c/°, cutoff 0.18 c/°; tonic firing: $k_c$ 12.4, $k_s$ 1.0, $r_c$ 2.6°, $r_s$ 22.9°, peak 0.029 c/°, cutoff 0.26 c/°; cell 3: burst firing: $k_c$ 5.2, $k_s$ 0.96, $r_c$ 3.8°, $r_s$ 12.4°, peak 0.041 c/°, cutoff 0.19 c/°; tonic firing: $k_c$ 4.3, $k_s$ 1.20, $r_c$ 3.5°, $r_s$ 14.0°, peak 0.04 c/°, cutoff 0.20 c/°. B: burst and tonic firing modes do not have different peak SFs. • and ○ show single cell, and are just as prevalent above the - - - of unity as below it. Inset: the means of the 2 distributions do not differ significantly (burst means ± SE: 0.033 ± 0.003 c/°; tonic: 0.032 ± 0.002 c/°; paired t-test, $P = 0.64$). Error bars show SE. C: spatial acuity is no different for burst and tonic spikes. • and ○ show 1 cell each and are evenly spaced about the - - - of unity. Inset: no significant difference between the means of the burst and tonic distributions (burst means ± SE: 0.17 ± 0.01; tonic: 0.16 ± 0.01; paired t-test, $P = 0.56$). All conventions as in B.]
responses sometimes differed in their relative amplitudes (though not consistently in favor of either burst or tonic firing), but were usually similar in shape. We included in our analysis all cells \((n = 61)\) in which the DoG function accounted for \(\geq 80\%\) of the variance of both burst and tonic raw data. In this sample, there were no significant differences in spatial tuning between the two response modes. Burst and tonic spikes fired to the same stimuli did not differ in terms of \(k_c\), the strength of the RF center (burst median: 7.59; tonic: 13.42; Wilcoxon matched pairs test, \(P = 0.43\)), \(k_s\), the relative strength of the RF surround (burst median: 0.98; tonic: 0.981; Wilcoxon matched pairs test, \(P = 0.38\)), \(r_c\), the radius of the RF center (burst median: 5.0°; tonic: 4.5°, Wilcoxon matched pairs test, \(P = 0.38\)), \(r_s\), the radius of the RF surround (burst median: 13.6°; tonic: 17.1°, Wilcoxon matched pairs test, \(P = 0.96\)), peak SF (burst means ± SE: 0.033 ± 0.003c/°; tonic: 0.032 ± 0.002c/°; paired \(t\)-test, \(P = 0.64\); Fig. 7B), or SF cutoff (burst means ± SE: 0.17 ± 0.01; tonic: 0.16 ± 0.01; paired \(t\)-test, \(P = 0.56\); Fig. 7C).

**Temporal frequency tuning**

Burst and tonic spikes differ with respect to their temporal tuning. Temporal frequency (TF) tuning was assessed by presenting drifting sinusoidal gratings of various TFs at optimal SF and 70% contrast. F1 response amplitudes of burst and tonic firing to these stimuli were then separately fitted with two-half-Gaussian equations (Eq. 3). These functions tended to be narrower, peak at lower TFs, and fall off at lower high TFs for burst versus tonic firing (Fig. 8A). We included in our
analysis all cells (n = 42) in which the two-half-Gaussians equation accounted for ≥80% of the variance of both burst and tonic raw data. In this sample, burst peak TFs were significantly lower than those of tonic spikes fired to the same stimuli (burst means ± SE: 3.9 ± 0.2 Hz; tonic: 4.5 ± 0.3 Hz; paired t-test, P = 0.007; Fig. 8B). Burst high50 values, taken as the high TF at which response amplitude dropped to half of its maximum, were also significantly lower than their tonic counterparts (burst means ± SE: 6.0 ± 0.3 Hz; tonic: 8.0 ± 0.5 Hz; paired t-test, P < 0.0001; Fig. 8C). To assess the tightness of TF tuning, a bandwidth measure was introduced. This measured, in octaves, the width of a TF tuning curve at half-maximum height. The bandwidth of burst spikes was significantly lower than that for tonic firing (burst means ± SE: 1.6 ± 0.1 octaves; tonic: 2.3 ± 0.2 octaves; paired t-test, P = 0.0002; Fig. 8D). However, the low-frequency roll-off of burst and tonic TF tuning curves was not different. The measure low50, the low TF at which a curve reached half its maximum height, did not differ significantly between burst and tonic activity (burst means ± SE: 2.2 ± 0.2 Hz; tonic: 1.8 ± 0.3 Hz; paired t-test, P = 0.25).

It is possible that some of the preceding differences in the temporal tuning of burst and tonic spikes are due not to fundamental differences between the two firing modes but instead to the stringent criteria used to identify burst spikes. Bursts as we define them must be preceded by a silent period of ≥100 ms (see METHODS). For this reason, spikes triggered by LTS activity might go undetected as bursts when, as is the case at high TFs, the period of sinusoidal grating stimuli approaches or is <100 ms. This is unlikely to affect any other data presented in this paper because stimuli in all non-TF tuning experiments were presented at 1 Hz or peak TF—always with a stimulus period well above 100 ms. However, it could affect our TF tuning results. We therefore investigated the effects of relaxing the requisite preburst silent period to 50 ms for stimuli with TF ≥8 Hz, following a precedent set by Sherman and colleagues (Guido et al. 1992; Lu et al. 1992). Surprisingly, this had remarkably little effect on burst-tonic differences in temporal tuning. With bursts preceded by ≥50-ms silence when stimulus TFs were ≥8 Hz, we still saw significant differences between burst and tonic spikes in high50 (burst means ± SE: 5.9 ± 0.3 Hz; tonic: 7.6 ± 0.4 Hz; paired t-test, P = 0.0003) and bandwidth (burst means ± SE: 1.6 ± 0.1 octaves; tonic: 2.3 ± 0.2 octaves; paired t-test, P = 0.0002), and there remained no burst-tonic difference in low50 (burst means ± SE: 2.0 ± 0.2 Hz; tonic: 1.6 ± 0.2 Hz; paired t-test, P = 0.16). In addition, response timing results, obtained using data collected during TF tuning experiments (see Fig. 5) were also unchanged: bursts were still phase-advanced (mean advance ± SE: 1.3 ± 0.2 rad; 1-sample t-test vs. 0, P < 0.0001) with a longer latency (burst median: 107 ms; tonic: 90 ms; Wilcoxon matched pairs test, P = 0.0008). The only different result noted when the preburst silent period was relaxed at high TFs was that peak TF was no longer significantly different between burst and tonic firing (burst means ± SE: 4.0 ± 0.3 Hz; tonic: 4.4 ± 0.3 Hz; paired t-test, P = 0.11). Note, however, that the trend for a higher TF peak in tonic firing remained. It therefore appears that the large majority, perhaps all, of burst-tonic differences in temporal tuning stem from fundamental differences in stimulus response, rather than from the ≥100-ms silence criterion used to identify burst firing in the present study.

### Contrast response characteristics

The contrast response functions of burst and tonic spikes were assessed by presenting drifting sinusoidal gratings of various contrasts at optimal SF. TF of these stimuli was either 1 Hz (18 cells) or optimal (14 cells). F1 response amplitudes of burst and tonic firing were then separately fitted with a hyperbolic function (Eq. 4) (Albrecht and Hamilton 1982) and measures were extracted from the portion of this curve between 0 and 100% contrast (Grubb and Thompson 2003). In most cases, both burst and tonic response amplitude grew rather gradually with increasing stimulus contrast (Fig. 9A1) or at least changed in a similar manner as contrast grew stronger (Fig. 9A3). In a few cases, though, a step-like function was found only in a cell’s burst responses (Fig. 9A2). Contrast gain was calculated as the slope of a tangent to the curve where response amplitude was 20% of its value at 100% contrast. We included in our analysis all cells (n = 32) in which the hyperbolic function accounted for ≥80% of the variance of both burst and tonic raw data. In this sample, a trend was observed toward higher gain values for tonic firing. This trend, however, was not significant (burst median: 0.26 spikes s⁻¹ · %⁻¹; tonic: 0.28 spikes s⁻¹ · %⁻¹; Wilcoxon matched pairs test, P = 0.06; Fig. 9B). Neither was a significant difference found between burst and tonic spikes in terms of cₕ, the contrast at which response amplitude reached 50% of its value at 100% contrast (burst means ± SE: 33.5 ± 3.2%; tonic: 36.8 ± 3.2%; paired t-test, P = 0.33; Fig. 9C). However, because in vitro experiments have suggested that the contrast response function for burst spikes might be more step-like than that of tonic spikes (McCormick and Feeser 1990; Ramcharan et al. 2000a; Zhan et al. 1999), the two firing modes were also compared with respect to the fitted curve parameter n (see Eq. 4). The larger this rate-of-change variable, the more step-like a curve is. n was significantly higher for burst spikes than for tonic spikes fired to the same stimuli (burst median: 3.34; tonic: 1.86; Wilcoxon matched pairs test, P = 0.0005; Fig. 9D).

### Signal detection

The more step-like contrast response functions of burst spikes (Fig. 9D), as well as their strong rectification (Fig. 6C), are consistent with the hypothesis that burst firing in an individual dLGN cell provides more information about whether a given stimulus is present than about that stimulus’ fine visual details (e.g., Sherman and Guillery 1996). In this hypothesis, burst spikes are primarily important for detecting visual stimuli. The relationship between burst firing and stimulus detection can be directly tested using receiver operating characteristic (ROC) analysis (Green and Swets 1966; Guido et al. 1995; MacMillan and Creelman 1991). This approach assesses how well a cell’s responses discriminate visual stimulation from periods of spontaneous activity, without ever employing a fixed detection criterion. This is achieved through the construction of ROC curves (Fig. 10A), which for a given stimulus plot the probability of observing different response sizes during spontaneous activity, P (false alarm), against the probability of observing those response sizes during visual
stimulation, \(P\) (hit) (see METHODS). The curve always runs from 
(0,0) to (1,1), and if real signal detection is occurring all points 
lie above the unity line. Area underneath the ROC curve (ROC
area) can then be used as a nonparametric measure of stimulus 
detection (Green and Swets 1966; Guido et al. 1995; MacMilla-

Given our experimental approach, we were unable to com-
pare ROC curves directly for burst and tonic spikes fired to the 
same stimuli. This can be readily done when responses are 
recorded intracellularly (Guido et al. 1995) but cannot be 
carried out with confidence when data are obtained from 
extracellular recordings. This is because the construction of 
ROC curves requires a direct comparison of spontaneously and 
visually driven activity, a comparison that is considerably 
complicated by recording periods in which no spikes are fired. 
During intracellular recordings, it is a simple matter to identify 
the underlying response mode of a neuron based on its mem-
brane potential. An intracellularly recorded lack of spontane-
ous firing, for example, can then be easily categorized as a lack 
of either burst or tonic firing and can be compared with a 
visually driven response (or lack of response) that occurred 
while the cell was in the same firing mode. When responses are 
recorded extracellularly, though, a response failure during, for 
example, visual stimulation, cannot similarly be compared with 
the appropriate period of spontaneously driven activity. With 
extracellular recording techniques therefore, we were not able 
to effectively compare spontaneous and visually driven activity 
in burst and tonic firing. Furthermore, we felt we could not

![Diagram of ROC curves](image-url)
simply ignore all stimulus cycles that produced a lack of burst or tonic activity because such cycles constituted a rather large proportion of all stimulus presentations: 75% of all stimulus cycles did not evoke a burst response, whereas 22% of all stimulus cycles did not evoke a single tonic spike.

However, we were able to assess the contribution of burst firing to stimulus detection more indirectly by asking whether the latter improved as the former became more prominent (cf. Guido et al. 1995). For each sinusoidal grating stimulus presented to each cell, we therefore calculated ROC area based on the total complement (burst + tonic) of the cell’s responses. Assigning response failures to a particular firing mode was therefore not an issue. We also calculated a “burst index” for each stimulus defined as the proportion of all stimulus cycles generating at least one burst response (Guido et al. 1995). The example ROC curves in Fig. 10A show that ROC area, and thus signal detection ability, tended to be larger when the burst index was higher. Indeed, over all stimuli presented to all cells, we observed a positive and significant correlation between ROC area and burst index (Spearman \( r = 0.59; P < 0.0001; n = 3,040 \)). This relationship was similarly positive and significant when assessed only for the optimal stimulus in each tuning experiment (Spearman \( r = 0.54; P < 0.0001; n = 339 \)), only for the nonoptimal stimuli in each tuning experiment (Spearman \( r = 0.58; P < 0.0001; n = 2,701 \)), or only for those stimuli associated with ROC areas \( > 0.5 \), representing significant stimulus detection (Spearman \( r = 0.54; P < 0.0001; n = 2,347 \)). The correlation between ROC area and burst index was also positive and significant if, instead of using one ROC area and one burst index per stimulus, we averaged ROC area and burst index values across all stimuli presented to each cell (Spearman \( r = 0.34; P = 0.0005; n = 102 \); Fig. 10B). Indirect evidence therefore suggests that burst firing is related to greater signal detection in the responses of mouse dLGN cells.

**Reliability in spike timing**

If burst firing tends to occur first in a dLGN cell’s responses to visual stimuli (Fig. 5), does it provide reliable information

**FIG. 10.** Levels of bursting correlate with stimulus detection in the responses of mouse dLGN cells. A: examples of receiver operating characteristic (ROC) analysis applied to all spikes (burst and tonic) in the responses of 3 individual mouse dLGN cells. Each plot is generated from the responses of a cell to a single sinusoidal grating stimulus. In each case here, the stimulus was nonoptimal, in that, of all stimuli presented during a tuning experiment, it did not produce the largest-amplitude F1 response. For a given spike count, 1 point in an ROC curve plots the probability \( P(\text{hit}) \) of attaining this count during a presentation of a visual stimulus vs. the probability \( P(\text{false alarm}) \) of attaining this count during an equal period of spontaneous activity. The area underneath the curve, expressed as a value between 0 and 1, then gives a nonparametric measure of a cell’s ability to distinguish visual from nonvisual stimulation (Guido et al. 1995) (see METHODS). ROC area values for each curve are shown in the bottom right of each plot, along with a burst index representing the percentage of stimulus cycles that produced \( > 1 \) burst response. In these 3 example stimulus presentations, it is clear that higher levels of bursting are coupled with better stimulus detectability. B: burst index values are positively and significantly correlated with ROC area values (Spearman’s \( r = 0.34; P = 0.0005, n = 102 \)). Although both measures were calculated for every stimulus presented to a given mouse dLGN cell (see A), the plot here shows a single burst index and ROC area for each cell, produced by averaging over all sinusoidal grating stimuli presented to that neuron. The positive and significant relationship is also observed when correlations are calculated over every sinusoidal grating stimulus, over all optimal stimuli, over all nonoptimal stimuli, and over all stimuli that produce ROC areas \( > 0.5 \), representing significant signal detection (see text).
concerning the time of stimulus onset? Although reliability in the absolute timing of spikes from stimulus onset cannot be assessed using the cyclic sinusoidal grating stimuli presented here, this question can be answered by taking a fixed point in the stimulus cycle and assessing reliability in the relative timing of spikes from that point. Reliability in burst and tonic spike timing was first assessed by calculating the mean deviation of spike times from the appropriate F1 response phase, normalized by the stimulus period (Eq. 6, Fig. 11A). Note that higher timing reliability here is reflected in lower values of the reliability measure. Over all grating stimuli, burst spikes occupy less of the stimulus period than tonic spikes (burst reliability median: 0.07; tonic: 0.13; Wilcoxon matched pairs test, \( P < 0.0001 \); Fig. 11B). An individual burst spike thus informs the brain more about stimulus timing than an individual tonic spike fired to the same grating. In addition, this advantage in timing reliability for burst spikes increases with increasing response size. While both burst and tonic spike times become less variable as overall F1 response size moves closer to optimal (burst Spearman \( r = -0.33, P < 0.0001, n = 2,178 \); tonic Spearman \( r = -0.53, P < 0.0001, n = 2,178 \)), the ratio of burst to tonic reliability also becomes lower (i.e., burst spikes are relatively less spread than tonic spikes) with increasing response optimality (Spearman \( r = -0.15, P < 0.0001, n = 2,178 \)).

In terms of the timing of the first spikes fired to grating cycles, however, tonic firing is more reliable than burst activity. First-spike reliability was measured by taking the SD of first-spike time latencies from the start of a half-cycle period centered on the burst or tonic F1 response phase, as appropriate.

![Figure 11](http://jn.physiology.org/)

**Figure 11.** Reliability of burst and tonic spike timing in mouse dLGN cells. A: assessing reliability in the timing of burst and tonic firing. Raster plots, in which each ○ represents 1 spike, show response timing for all burst and tonic spikes fired to a drifting sinusoidal grating (A1) or for just the 1st burst and tonic spikes fired to each cycle of the stimulus (A2). Note the close superposition of burst spikes in A1 that cause this plot to look very similar to the 1st-spike plot in A2. In A1, the vertical line represents the phase of the burst or tonic F1 component, as appropriate. Reliability values, displayed at bottom left, represent the mean deviation of spike times from this line, normalized by the stimulus period. Note that higher timing reliability is reflected in lower values of this measure. In A2, ---, the start of the half stimulus period centered on the phase of the burst or tonic F1 component, as appropriate. First-spike reliability values, shown at bottom right, represent the SD of 1st-spike time distances from this line (see METHODS). Again, higher timing reliability is reflected in lower values of this measure. As reflected in these values, burst spikes show more reliability in timing when all spikes of a particular firing mode are taken into consideration. However, when just the first spikes fired to each stimulus cycle are analyzed, there is very little difference in timing reliability between burst and tonic firing. B: over all spikes, burst timing is more reliable. •, which each represent a single sinusoidal grating stimulus, are concentrated above the - - - unity line. Tonic spikes thus occur over a larger portion of a cycle than burst spikes fired to the same stimulus. This is confirmed in the inset, which shows that the burst and tonic firing median reliability values are significantly different (burst median: 0.07; tonic: 0.13; Wilcoxon matched pairs test, \( P < 0.0001 \)). C: the 1st tonic spikes fired to each stimulus cycle are more reliable in their timing than the 1st burst spikes fired to the same stimuli. Burst and tonic 1st-spike reliability values appear well correlated with each other, and • appear rather evenly distributed about the unity line. Nevertheless, the inset shows that median 1st-spike reliability value for tonic firing is significantly smaller than that for burst spikes (burst median: 70 ms; tonic: 64 ms; Wilcoxon matched pairs test, \( P < 0.0001 \)). Conventions as in B. D: burst and tonic spike timing becomes more reliable with increasing stimulus contrast. The plot shows median reliability values for each stimulus contrast, averaged over all contrast response experiments. E: burst and tonic 1st-spike timing also becomes more reliable with increasing stimulus contrast. Conventions as in D.
(Eq. 7, Fig. 11A). Again, higher timing reliability is reflected in lower values of this reliability measure. Although this measure produced very similar reliability estimates for tonic and burst firing (e.g., Fig. 11A), over all grating stimuli tonic first spikes were less jittery in time than burst first spikes fired to the same stimuli (burst median: 70 ms; tonic: 64 ms; Wilcoxon matched pairs test, $P < 0.0001$; Fig. 11C). This reliability advantage of tonic firing, however, decreased as stimuli became more optimal. First-spike reliability values for both burst and tonic firing decreased as overall F1 response amplitudes approached those produced by optimal stimuli (burst Spearman $r = -0.45$, $P < 0.0001$, $n = 1,283$; tonic Spearman $r = -0.48$, $P < 0.0001$, $n = 1,283$), but the ratio of burst to tonic first-spike reliability values also decreased (i.e., burst 1st-spike reliability became relatively better) with increasing stimulus optimality (Spearman $r = -0.19$, $P < 0.0001$, $n = 1,283$). So, although tonic first spikes provide more accurate information concerning stimulus “onset,” this advantage over burst first spikes becomes less marked for more optimal stimuli.

This relationship between response strength and reliability in spike timing was investigated in more detail for contrast response characteristics because of a specific prediction arising from an in vitro electrophysiological study. Zhan et al. (1999) found that the latency of the first burst action potential caused by a low-threshold calcium spike was longer after weaker depolarizing current injections. Presumably assuming that longer latencies are associated with greater variability in response timing, these authors predicted that increasing stimulus contrast in vivo would be met with increased reliability in burst spike timing. Indeed, the present data show that this is the case. Across all stimuli in all contrast experiments, stimulus contrast is negatively and significantly correlated with burst reliability (Spearman $r = -0.22$, $P < 0.0001$, $n = 652$) and burst first-spike reliability (Spearman $r = -0.29$, $P < 0.0001$, $n = 405$; see Fig. 11, D and E). And within contrast experiments, burst reliability and first-spike reliability values were both higher for the highest contrast for which they could be measured (reliability: low contrast mean $\pm$ SE: 0.11 $\pm$ 0.01; high contrast: 0.07 $\pm$ 0.007; paired t-test, $P < 0.0001$; 1st-spike reliability: low contrast mean $\pm$ SE: 126 $\pm$ 15 ms; high contrast: 100 $\pm$ 15 ms; paired t-test, $P = 0.01$). However, increasing reliability with increasing stimulus contrast is not a feature restricted only to burst firing. Across all contrast stimuli, the variability of tonic spike timing also decreased with increasing contrast (reliability: Pearson $r = -0.43$, $P < 0.0001$, $n = 691$; 1st-spike reliability: Pearson $r = -0.34$, $P < 0.0001$, $n = 792$; see Fig. 11, D and E). Within contrast experiments, too, the highest contrasts for which reliability values could be measured produced significantly more reliable tonic spike timing than the lowest contrasts for which these values could be obtained (reliability: low contrast median: 0.20; high contrast: 0.11; Wilcoxon matched pairs test, $P < 0.0001$; first-spike reliability: low contrast mean $\pm$ SE: 154 $\pm$ 11 ms; high contrast: 70 $\pm$ 7 ms; paired t-test, $P < 0.0001$).

Thus although the reliability of burst spike timing increases as stimulus contrast increases, as predicted by Zhan et al. (1999), tonic firing reliability becomes better with increasing contrast as well.

Analyses treating bursts as unitary events

In the preceding analyses, visual response properties are compared between spikes that form part of bursts and spikes that do not form part of bursts. We extended these analyses by asking what happens if instead of treating bursts as events made up of component spikes, bursts are analyzed as unitary events. In other words, are the same burst-tonic functional differences (and similarities) observed if we analyze the response properties, not of burst spikes, but of bursts per se? To address this issue, bursts were treated as unitary events, occurring at the poststimulus time of their first spike. Using this approach, we found an almost identical pattern of burst-tonic functional relationships to that outlined in the spike-based analyses described in the preceding text. As was the case when burst and tonic spikes were compared, burst events, in terms of bursts per second, were not significantly different ($P > 0.05$) from tonic spikes with respect to linearity of spatial summation, spatial frequency tuning, and contrast $c_{50}$. Also in line with the preceding analyses, bursts as unitary events had higher visual than spontaneous firing rates, had advanced response phases but longer latencies than tonic firing, were more rectified than tonic spikes, had smaller ROC areas than tonic spikes, had more step-like contrast response curves than tonic firing, and, in their TF responses, had lower peak, high50, and bandwidth values (all $P < 0.05$). The only difference between analyses based on burst spikes versus those based on bursts per se occurred for the TF tuning measure low50—the low TF at which responses fell to half their maximal height. While burst spikes show a nonsignificant trend toward higher low50 values than tonic spikes (see preceding text), bursts as unitary events do possess significantly higher low50 values compared with tonic firing (burst median: 1.8 Hz; tonic: 1.4 Hz; Wilcoxon test, $P = 0.03$). This very small difference between two separate ways of assessing burst response characteristics suggests that the burst-tonic functional differences outlined in the preceding text are rather robust phenomena.

Burst-tonic differences in on-center and off-center cells

In our previous description of visual response properties in the mouse dLGN (Grubb and Thompson 2003), we looked for evidence of parallel processing of visual information by different cell types and found it only in functional differences between cells that respond primarily to increases in stimulus luminance (on center) and cells that respond primarily to decreases in stimulus luminance (off center). It was therefore worth asking whether the burst-tonic differences in visual processing described in the preceding text occurred predominantly and/or consistently in either of the two main RF types in the dLGN.

For those response property parameters, which were normally distributed or which could be rendered normal by simple logarithmic transforms, we employed repeated-measures ANOVA tests, in which firing mode (burst/tonic) was the within-subject variable and center type (on-off) was the between-subject variable. Any significance in the firing mode $\times$ center type interaction would then imply that a given burst-tonic difference was significantly stronger in cells of one or
other center type. This ANOVA analysis was possible for 16 response parameters: latency, phase, linearity, rectification, \( r_c \), \( r_s \), SF peak, SF cutoff, TF peak, \( \text{high}_{50} \), bandwidth, \( \text{low}_{50} \), contrast gain, \( c_{50} \), and contrast \( n \). In most cases, the firing mode \( \times \) center type interaction was not significant \( (P > 0.05) \). In four cases, however, the interaction was significant \( (P < 0.05) \), suggesting that burst-tonic differences in these parameters were stronger in either ON- or OFF-center neurons. Burst-tonic differences in response phase and rectification were significantly larger in ON-center cells, while differences between the two firing modes in latency and \( \text{high}_{50} \) were significantly greater in OFF-center cells. Still, the fact that burst-tonic comparisons were not significantly different between ON- and OFF-center cells for the majority of visual response measures, coupled with the observation that the four significant effects described in the preceding text were split evenly between ON- and OFF-center neurons, show that the burst-tonic differences described in the present paper are not due primarily to firing mode distinctions within cells of one single center type.

This conclusion is backed by consideration of those parameters that could not be rendered normally distributed by simple transforms. These response measures were compared by simply noting the results of paired burst-tonic comparisons applied to ON- and OFF-center data separately. We found positive and significant correlations between ROC area and burst index in both ON- and OFF-center neurons, observed that spike timing reliability is significantly better in the burst spikes fired by both ON- and OFF-center cells and noted that both center types possess no significant difference between burst and tonic spikes with respect to the RF surround parameter \( k_s \). Only in the remaining 2 response measures did burst-tonic differences appear to be dominated by cells of one particular center type. Like our dLGN sample as a whole, ON-center cells showed a significantly greater percentage of burst spikes in visually driven versus spontaneous firing. OFF-center cells, however, did not. Conversely, while OFF-center cells mirrored our overall results in that their burst first-spike reliability was significantly worse than that of tonic spikes, this effect was not significant in ON-center cells. Still, one burst-tonic difference due mainly to the responses of ON-center cells, and one due mainly to the responses of OFF-center cells, along with the ON-OFF similarities described in the preceding text, suggests that, overall, burst-tonic differences in the visual response properties of mouse dLGN cells are not due predominantly to the responses of cells of a single center type.

Why are burst spikes functionally different from tonic spikes?

Bursts possess two properties that distinguish them from the large majority of tonic spikes: high-frequency firing and a long preceding silent period. The possibility exists that the differences between burst and tonic spikes outlined in the preceding text might be due primarily to one or other of these distinguishing features—for example, differences in temporal tuning could be caused purely by the long silent period required for burst spikes to occur rather than being caused by LTS activity per se. To investigate this possibility, we compared the visual response properties of burst spikes with three types of tonic responses, all identified, like burst spikes themselves (see METHODS), on the basis of temporal patterns in spike firing. High-frequency tonic (HFT) spikes were separated by short interspike intervals of \( \approx \) 4 ms, but, unlike bursts, groups of HFT spikes were not preceded by \( \approx 100 \) ms of nonspiking dead time. In contrast, gap spikes were preceded by \( \approx 100 \) ms of silence but unlike bursts had following ISIs of \( > 4 \) ms. The final group, TX spikes, comprised the remainder of nonburst firing, and represented normal tonic activity (Fig. 12A). By comparing the visual response properties of these different types of activity, we hoped to glean some understanding of the underlying reasons for burst-tonic functional differences. For instance, the 4-way analysis might find that in the majority of cases where burst spikes differ from tonic spikes, HFT spikes differ from TX spikes in the same direction as the burst-tonic difference, but gap spikes do not. This might then indicate that the functional distinctions between burst and tonic responses outlined above are due mainly to the high-frequency firing component of bursts, rather than the requisite preceding silent period.

The results of the 4-way comparisons are shown in Fig. 12B. For each visual response measure, we employed a repeated-measures ANOVA or Friedman test (depending on normality) followed by appropriate posttests (Tukey or Dunn, depending on normality) to assess the significance of differences between the four spike types. Our main observation is that the burst-tonic differences reported in the main body of paper are not as a whole attributable to either the high-frequency firing or the preceding silence features of burst firing because the relationships between HFT, gap, and TX spikes are not consistent. There were nine visual response measures reported in the preceding text in which burst and tonic firing differed significantly. In three of these (rectification, bandwidth, 1st-spike timing reliability), both HFT and gap responses were significantly different from TX spikes in the same direction as the burst-TX difference (Fig. 12B, 3, 6, and 9). This might suggest that the original burst-tonic differences in these parameters were due to both high-frequency firing and preburst silence. However, there were two cases (latency, phase) in which gap spikes and TX spikes differed significantly and in the same direction as the burst-TX difference, but no significant difference was seen between HFT and TX firing (Fig. 12B, 1 and 2). Trends in the contrast \( n \) parameter were also in this direction, although sample sizes were small and differences were insignificant (Fig. 12B7). In these parameters, the original burst-tonic differences appear to be mainly due to burst firing’s feature of preceding silence. This is immediately contradicted, though, by the case of spike timing reliability, in which HFT and TX spikes differ in same way as burst and TX spikes, but where the gap-TX difference is significant in the opposite direction (Fig. 12B8). For this parameter, high-frequency firing seems to be the main contributor to the burst-tonic difference reported above. Finally, there were two parameters in which the four-way comparisons revealed a rather puzzling result. TF peak and \( \text{high}_{50} \) measures did not differ between burst and TX spikes; instead, the original burst-tonic difference appeared to be made entirely by gap spikes’ contribution to tonic sample averages, since gap responses differed significantly from all other firing types (Fig. 12B, 4 and 5).

These results show that it was not possible to attribute burst-tonic functional differences, as a whole, to a single feature of burst firing. Perhaps assessing the firing properties of different subgroups of tonic spikes is not the best approach to
this problem, but it was a sensible first step. Maybe intracellular recordings, by allowing a more accurate classification of burst spikes from their tonic counterparts, is produced by a complex combination of both high-frequency firing and a requisite preceding silent period.

**DISCUSSION**

We present a comprehensive comparison of the visual response properties of burst and tonic firing in mouse dLGN cells. Once burst-like events in the firing of mouse dLGN neurons had been identified on the basis of temporal patterns of spike firing, various features of these events were shown to match those previously described for thalamic bursting in other species and preparations. Burst spikes were shown to occur more often during visual stimulation than during periods of nonburst firing and represented “normal” tonic activity. ISI plots (see Fig. 1) were generated for each group of spikes. The line marked TF displays the temporal period of the stimulus (here 6 Hz). All other conventions as in Fig. 1. B: average values for burst (B), TX, HFT, and gap (G) spikes are shown for the 9 response measures in which significant burst-tonic differences were found in our original analyses. In cases where all 4 samples were normally distributed, bar plots show the means ± SE (2, 4, 5, 6); in all other cases, plots show medians (1, 3, 7, 8, 9). The P values at top left show the result of paired comparisons across the 4 spike types (repeated-measures ANOVA or Friedman tests, depending on normality). When P < 0.05, there was significant variation among groups. The results of individual post hoc comparisons (Tukey or Dunn, depending on normality) are shown above the appropriate bars in cases where the overall repeated-measures test produced significant results. Only the results of B-TX, TX-HFT, and TX-G comparisons are shown because these are the most informative and are discussed in the text. ns, nonsignificant; *, P < 0.05; ** P < 0.01; *** P < 0.001. It is clear that relationships between burst, TX, HFT, and gap spikes are complex and do not follow similar patterns across all response parameters. The relationships between burst and TX spikes are not always mirrored in either the relationships between TX and HFT spikes, or the relationships between TX and gap spikes. Neither the high-frequency firing component of burst spikes nor the requisite preceding silence of bursts can therefore be wholly responsible for the burst-tonic functional differences described in the preceding text.

**FIG. 12.** Assessing possible causes of burst-tonic functional differences in the mouse dLGN. A: in 4-way analyses, burst firing was compared with 3 distinct subgroups of tonic spikes. High-frequency tonic (HFT) spikes were separated by short interspike intervals of ≤4 ms, but, unlike bursts, each cluster of HFT spikes was not preceded by ≥100 ms of nonspiking dead time. "Gap" spikes were preceded by ≥100 ms of silence, but unlike bursts had following ISIs of >4 ms. TX spikes comprised the remainder of nonburst firing, and represented "normal" tonic activity. ISI plots (see Fig. 1) were generated for each group of spikes. In the example shown, all spikes were produced by a single mouse dLGN cell during a contrast tuning experiment (see METHODS). SF and TF were optimal. Please note that, although the large number of high-frequency spikes fired by the example cell means that certain spikes appear to be shared between burst and HFT firing, these spike groups actually represent mutually exclusive response subsets. The line marked TF displays the temporal period of the stimulus (here 6 Hz). All other conventions as in Fig. 1.
spontaneous activity. Burst and tonic spikes within individual mouse dLGN neurons were then compared with respect to their response timing, linearity, SF and TF tuning, contrast response characteristics, signal detection properties, and spike time reliability. This analysis uncovered a number of functional distinctions between the two firing modes.

Comparison with previous studies

INCIDENCE OF BURSTING. Criteria previously used to identify burst firing from extracellular recordings in the cat dLGN (e.g., Guido et al. 1992; Lu et al. 1992) were used successfully here in mouse dLGN neurons to classify firing events that already showed distinct temporal patterns (Fig. 1) and that possessed properties similar to those reported for burst firing in other species and preparations (Fig. 2). Coupled with in vitro thalamic recordings showing that \( I_T \) and burst spikes are very similar across different mammalian species (guinea pig: Jahnsen and Llinas 1984; McCormick and Feeser 1990; cat: Zhan et al. 1999; monkey: Ramcharan et al. 2000a; mouse: Kim et al. 2001), these data suggest that burst firing is well conserved across the mammalian order. It will require intracellular recordings from mouse dLGN cells in vivo, however, for us to know for sure that the action potentials classified as burst firing in this study really were riding on a low-threshold calcium spike.

In terms of burst prevalence, \( \approx 20\% \) of spikes form part of bursts in the anesthetized mouse dLGN during presentation of sinusoidal grating stimuli (Fig. 4). This is comparable to the value of 20–25\% reported for X cells during visual stimulation in the anesthetized cat (Lesica and Stanley 2004; Reinagel et al. 1999), but much lower than the Y cell value under the same conditions (60\%) (Reinagel et al. 1999), and much higher than the 1–2\% reported during visual stimulation in the awake monkey dLGN (Ramcharan et al. 2000b). Bursting was likely facilitated by the anesthetized preparation employed here (cf. Guido and Weyand 1995; Reinagel et al. 1999). If this is the case, experiments in anesthetized animals may not produce results that can translate directly to behavioral situations but do present the best opportunity to compare the response properties of many burst and many tonic spikes fired by the same cells to the same sensory stimuli.

RELATIVE SPIKE TIMING. Burst spikes in the mouse dLGN have longer latencies, but because they are triggered by earlier stimulus phases tend to occur before tonic spikes in responses to sinusoidal gratings (Fig. 5). The situation in the cat dLGN is almost identical. Cells that fire more burst spikes have longer latencies and larger phase advances (Mukherjee and Kaplan 1995), while stimulating the histaminergic tuberomammillary nucleus increases tonic firing and produces a phase lag in responses to grating stimuli (Uhlrich et al. 2002). Furthermore, a Fourier analysis of in vitro responses to sinusoidal inputs shows that the line relating TF to F1 response phase is steeper (indicative of longer latency), with a higher \( y \)-intercept (indicative of a phase advance), for burst versus tonic spikes (Smith et al. 2000). And although Guido et al. (1992) report that burst and tonic spikes in the cat dLGN have similar latencies, with burst spikes phase advanced, close inspection of their raw data reveals that their plots of TF versus F1 response phase are strikingly similar to those shown in Fig. 5 here: burst spikes are phase advanced at low but not high TFs. Had line fitting procedures not differed between the two studies, with Guido et al. (1992) employing response-weighted fits that decrease the influence of weak responses to high TFs, both might have reported longer latencies for burst spikes.

The phase advance and the longer latency of burst spikes could be consequences of the LTS that produces burst firing (Jahnsen and Llinas 1984). By definition, the LTS is low threshold, and there is in vitro evidence that burst spikes can be elicited by smaller stimulation amplitudes than tonic spikes (e.g., Zhan et al. 1999). In vivo, smaller stimulation strengths, in the form of low rates of firing in retinal afferents, should occur at lower stimulus contrasts and therefore at earlier phases of the stimulating half cycle (dark or bright) of a sinusoidal grating. Burst spikes would thus be the earliest spikes triggered by a given cycle of such a stimulus. Long latencies, on the other hand, could be a consequence of the LTS’s slow dynamics. In vitro, burst spikes require longer durations of stimulation than tonic spikes (McCormick and Feeser 1990), and in vivo, retinal spikes preceding burst firing occur much longer before geniculate activity than retinal spikes preceding tonic firing (Rowe and Fischer 2001).

Linearity

Burst and tonic spikes in the mouse dLGN do not differ with respect to linearity of spatial summation, but burst firing is more rectified than tonic activity (Fig. 6). This is the first study to use a null test (Enroth-Cugell and Robson 1966; Hochstein and Sharpley 1976) to assess linearity of spatial summation in the two thalamic firing modes. Because both burst and tonic spikes display linear summation in linear mouse dLGN cells, it would be extremely interesting to know whether the ON-OFF doubling nonlinearity of cat Y cells (e.g., So and Sharpley 1979) is carried by burst spikes, tonic spikes, or both.

In contrast, rectification nonlinearities have been well studied previously. As in the mouse dLGN, burst spikes in the cat dLGN in vivo (Guido et al. 1992; Lu et al. 1992; Mukherjee and Kaplan 1995) and in vitro (Smith et al. 2000) follow the temporal form of a sinusoidal stimulus less closely than tonic spikes.

Spatial and temporal tuning

Burst and tonic firing in the mouse dLGN do not differ in terms of SF tuning (Fig. 7). No other studies have directly compared SF tuning in the two firing modes, although Guido et al. (1992) did compare the percentage of burst spikes fired to different stimuli across cells and found no variation with SF. In addition, stimulation of the tuberomammillary nucleus, which increases tonic firing in the cat dLGN, produced very little change in the SF tuning properties of geniculate neurons (Uhlrich et al. 2002). Those small but significant changes that were produced—increased RF center strength \( k_c \) and decreased RF center radius \( r_c \)—could well have been produced by nonburst-related effects of the stimulation but were nevertheless mirrored by nonsignificant trends in the mouse dLGN data. In reverse correlation studies of the spatial RF structure of burst and tonic spikes, initial results are inconsistent: whereas Rivadulla et al. (2003) find RF center size to be smaller for burst responses, Allitto et al. (2003) do not but do observe
increased surround strength during burst firing. There are therefore no large or consistent differences in the spatial response properties of burst and tonic spikes.

Burst spikes in the mouse dLGN prefer lower TFs and show sharper TF tuning than tonic spikes (Fig. 8). Because burst firing can only occur after \( \geq 100 \) ms of hyperpolarization, one might expect it to have difficulty in following higher TFs. Indeed, in vitro studies in the guinea pig (McCormick and Fezer 1990) and cat (Smith et al. 2000) also report that burst spikes stop responding at lower cutoff TFs than tonic spikes. The present finding of sharper TF tuning (i.e., lower bandwidth) in burst firing is also well supported by previous data. Tonic TF tuning in slices taken from the cat dLGN is more broadband than its burst counterpart (Smith et al. 2000), while an increased surround strength during burst firing. There are some slight disagreement between the present data and the latter in vivo cat study, however. While burst spikes here displayed sharper TF tuning mainly by dint of reduced responses at high TFs, cat dLGN cells showed sharpened tuning associated with bursting because of reduced responses at low TFs (Mukherjee and Kaplan 1995). This may reflect species or methodological differences between the two studies but could also be caused by random fluctuations between different samples. There was certainly a trend toward higher \( \text{low}_{30} \) values for burst firing in the mouse dLGN.

**Contrast response characteristics**

Comparison of contrast response characteristics showed that burst and tonic firing modes do not differ in terms of contrast gain or \( c_{50} \). However, the contrast-response curves of burst firing are more step-like (reflected in higher values of the parameter \( n \)) than those of tonic spikes (Fig. 9). This latter difference might have been predicted on the basis of in vitro investigations. Due to the all-or-nothing nature of the LTS, the amplitude of which depends not on the strength of depolarizing inputs but rather on the relative hyperpolarization of the membrane potential when those inputs arrive, the burst response of a dLGN cell to increasing current injection at a constant membrane potential resembles a step function. Tonic firing, in contrast, increases almost linearly with increasing stimulation strength (McCormick and Fezer 1990; Ramcharan et al. 2000a; Zhan et al. 1999). Burst contrast-response functions observed in vivo may not be entirely step-like, and in vivo tonic functions are certainly not always completely linear (see Fig. 9A). Nevertheless, the relatively more step-like relationship between burst responses and stimulus contrast might be expected based on the properties of the LTS and represents a significant functional difference between burst and tonic firing.

**Stimulus detection**

The preceding discussion shows that differences in burst and tonic visual coding in the mouse dLGN usually reflect similar differences reported in the cat dLGN. The functions of the two firing modes therefore seem rather well conserved across these two mammalian species. This conclusion is bolstered by our analysis of stimulus detection: in the mouse, as in the cat (Guido et al. 1995), better signal detection is associated with more prominent burst firing in a dLGN cell’s responses (Fig. 10). It appears, however, that bursting may not be associated with better stimulus detection in all thalamic nuclei (or maybe in all species), given that a greater incidence of burst firing is not related to better stimulus detection in the guinea pig auditory thalamus (Massaux et al. 2004).

**Reliability of spike timing**

In the mouse dLGN, the relative timing of all burst spikes is more reliable than that of all tonic spikes, but tonic firing is more reliable in terms of the first spikes fired to a stimulus (Fig. 11). A single burst spike therefore carries more information concerning the timing of a stimulus event than a single tonic spike. Combined with the fact that burst spikes occur earlier than tonic spikes in responses to a given stimulus, this may make bursting ideal for signaling to cortex when a stimulus appeared, even if the first spikes in bursts are not quite so reliable in their timing as the first spikes in tonic responses. This latter result contradicts a finding in the cat dLGN, where the reliability of first-spike timing was better for burst than for tonic responses (Guido and Sherman 1998). This contradiction may reflect true species differences, possible differences in halothane anesthetic depth (see preceding text), or differences in the stimuli employed in the two studies. Unlike the flashed spots employed by Guido and Sherman (1998), the sinusoidal stimuli used here do not possess a single onset time and therefore might not be entirely appropriate for assessing spike timing properties. Still, the increased variability in the timing of burst first spikes in the mouse dLGN does appear to fit well with a different cat study, in which paired retinal and dLGN recordings showed burst spikes to be far more loosely and variably coupled to retinal inputs than tonic spikes (Rowe and Fischer 2001).

Although the main focus of this paper was simply to describe the different types of visual information that burst and tonic spikes in the mouse dLGN might send to visual cortex, it is worth briefly considering some implications of the mechanism by which burst and tonic stimulus selectivity arises in the first place. Tonic firing in thalamocortical relay cells is largely determined by excitatory retinal inputs with the correspondence rather tight between presynaptic spikes in retinal ganglion cells and postsynaptic spikes in geniculate neurons (Rowe and Fischer 2001). The properties of the \( I_T \) current underlying burst responses, however, mean that although burst spikes are normally triggered by excitatory retinal inputs, their stimulus selectivity should be largely determined by local inhibitory circuitry. Not only is membrane hyperpolarization needed to de-inactivate \( I_T \) and allow burst firing to occur (e.g., Jahnsen and Linas 1984), the degree of membrane hyperpolarization also determines the amplitude of burst responses: the more hyperpolarized the membrane, the more burst spikes result from a constant excitatory trigger (Zhan et al. 1999). Measures of F1 response amplitude, such as those widely employed to assess stimulus selectivity in this paper and elsewhere, depend on both the probability of a response occurring to a particular stimulus cycle and the size of that response when it does occur. In burst firing, both of these factors will be largely determined by the degree of hyperpolarization present in dLGN cells before they receive an excitatory triggering input from the retina. It follows that burst spike response selectivity during the presentation of drifting...
What do bursts do?

The analyses presented here extend our knowledge of the visual encoding properties of burst and tonic firing in the dLGN and will be important when future studies investigate the molecular basis of thalamic processing using mutant mice (see Introduction), but do they shed any light on the fundamental functions of the two firing modes? They certainly confirm that tonic spikes are used to extract detailed information about visual stimulus attributes. Where tuning properties differ between burst and tonic firing, tonic spikes, with less rectification, broader TF tuning, better responsivity to high TFs, and more linear responses to increasing contrast, are better placed to signal information about a wider range of visual stimulus attributes. But it is not at all clear from the present analysis what the role of burst firing might be.

The prevailing hypothesis of burst function revolves around previous reports of greater rectification and signal detection in burst firing (e.g., Guido et al. 1992; Sherman 2001; Sherman and Guillery 1996). Burst firing is seen as a “wake-up call” for cortex, detecting stimuli but then leaving any detailed analysis to tonic firing. Indeed, the increase in stimulus detection that occurs with increased burst prominence in mouse dLGN cells (Fig. 10), along with the phase advance of burst firing (Fig. 5) (Guido et al. 1992; Mukherjee and Kaplan 1995) and the reliable timing of burst spiking as a whole (Fig. 11) suggest that burst spikes may be ideal for signaling to cortex (Swadlow and Gusev 2001; Swadlow et al. 2002) that a stimulus has occurred and that it started a certain time ago. Furthermore, given that the visual encoding differences between tonic and burst spikes are not huge (Figs. 5–8), this bursting detection signal could also contain a great deal of information concerning stimulus attributes, something for cortex to start to process before more detailed tonic spike-carried information arrives. In awake cats, burst spikes are fired at the very start of stimulus presentations and visual fixation periods (Guido and Weyand 1995; Weyand et al. 2001), suggesting that bursting may indeed signal the presence of new stimuli to cortex. In addition, burst spikes fired by X cells in the anesthetized cat dLGN tend to occur when excitatory features follow the prolonged presence of inhibitory features in natural movie stimuli (Lesica and Stanley 2004). In natural scenes, therefore bursts might encode the appearance of new objects. However, burst firing is also more prevalent during “passive” viewing in awake cats (Weyand et al. 2001) and, when occurring before stimulus onset, increases the probability of a subsequent stimulus-induced response in rat somatosensory thalamus (Fanselow et al. 2001). This suggests that burst spikes may not be detecting stimuli per se but are instead priming the brain to do so. It would be extremely useful to know whether bursting during stimulus presentation correlates with the animal’s detection of that stimulus.

Another view of firing mode function, based on the different temporal tuning properties of burst and tonic spikes (Mukherjee and Kaplan 1995) (Fig. 8), argues that the thalamus is a “tuneable filter” for visual information. Various modulatory inputs to the dLGN, by altering the probability of burst firing in relay cells (e.g., Godwin et al. 1996; Uhrlich et al. 2002), could alter the tuning of information sent to visual cortex. Given the present data this could certainly work in the contrast (Fig. 9) and TF (Fig. 8) domains, although it is unlikely to apply to spatial information (Fig. 7). However, it is unclear to what extent bursting tuning properties are functional adaptations and to what extent they are simply artifacts of burst properties that might have evolved for different reasons. It certainly seems strange that so much circuitry would be invested into changing temporal tuning in the dLGN when such a large amount of temporal information is discarded at the geniculo-cortical synapse (e.g., Hawken et al. 1996). And while increasing bursting might tighten temporal tuning in the dLGN, this would necessarily occur at the expense of detailed information about stimulus contrast (Fig. 9) and stimulus temporal form (increased rectification; Fig. 6). Still, until a definitive test of this hypothesis is formulated, it remains a valid suggestion.

Finally, there is a view that burst firing might be vital not for signaling specific stimulus attributes but for synchronizing activity across thalamic neurons. Sillito et al. (1994) showed that synchronous activity in dLGN cells is dependent on cortical feedback. In a model of these data, Kirkland and Gerstein (1998) show that this cortically induced synchronization is critically dependent on burst firing. The reliability of burst timing (Fig. 11) might be useful in this regard, although direct tests of the hypothesis, and a better understanding of whether bursts are linked more strongly to changes in sensory stimuli or changes in internal state, await multi-unit thalamic recordings.

Acknowledgments

The authors thank D. Smyth for computational assistance and C. Akerman for helpful discussions. M. S. Grubb was a student on the Wellcome Trust 4-year PhD program in Neuroscience.

References


