*Visual Neuroscience* (2000), **17**, 55–62. Printed in the USA.


# Burst and tonic firing in thalamic cells of unanesthetized, behaving monkeys

EION J. RAMCHARAN, JAMES W. GNADT, AND S. MURRAY SHERMAN

Department of Neurobiology, State University of New York, Stony Brook

(RECEIVED April 2, 1999; ACCEPTED August 2, 1999)

**Abstract**

Thalamic relay cells fire in two distinct modes, burst or tonic, and the operative mode is dictated by the inactivation state of low-threshold, voltage-gated, transient (or T-type) $Ca^{2+}$ channels. Tonic firing is seen when the T channels are inactivated *via* membrane depolarization, and burst firing is seen when the T channels are activated from a hyperpolarized state. These response modes have very different effects on the relay of information to the cortex. It had been thought that only tonic firing is seen in the awake, alert animal, but recent evidence from several species suggests that bursting may also occur. We have begun to explore this issue in macaque monkeys by recording from thalamic relay cells of unanesthetized, behaving animals. In the lateral geniculate nucleus, the thalamic relay for retinal information, we found that tonic mode dominated responses both during alert behavior as well as during sleep. We nonetheless found burst firing present during the vigilant, waking state. There was, however, considerably more burst mode firing during sleep than wakefulness. Surprisingly, we did not find the bursting during sleep to be rhythmic. We also recorded from relay cells of the somatosensory thalamus. Interestingly, not only did these somatosensory neurons exhibit much more burst mode activity than did geniculate cells, but bursting during sleep was highly rhythmic. It thus appears that the level and nature of relay cell bursting may not be constant across all thalamic nuclei.

**Keywords:** Vision, LGN, Sleep, Low-threshold spike, Burst

## Introduction

Thalamic relay cells fire in two different response modes, burst and tonic, and we can assume that the two firing modes will differentially affect the relay of information from the external world to the cortex. The switch between modes for thalamic cells depends on the inactivation state of their voltage-gated, T-type $Ca^{2+}$ channels. These channels are inactivated at depolarized membrane potentials, so that an activating input from more depolarized levels, if large enough, will activate a sustained train of unitary action potentials. This is tonic firing. However, the inactivation of the T channels is removed by sustained hyperpolarization (i.e. the T channels are de-inactivated), and thus an activating input from relatively hyperpolarized levels activates the channels, giving rise to an inward $Ca^{2+}$ current ($I_T$). As a result, a low-threshold $Ca^{2+}$ spike is produced (Jahnsen & Llinàs, 1984*a*,*b*; Steriade & Llinàs, 1988). A sufficiently large low-threshold spike results in the production of a high-frequency cluster of action potentials characteristic of burst mode.

In a behavioral context, it is somewhat unclear when one or the other of these modes become the dominant firing pattern. It had been thought that relay cell bursting is seen only during certain phases of sleep, deep anesthesia, or absence seizures, when the bursting is rhythmic (Steriade et al., 1993). Because of the prevalence of rhythmic bursting during non-awake states, it was suggested that this mode of firing acts to interrupt the thalamic relay of sensory signals (Livingstone & Hubel, 1981; Steriade & McCarley, 1990; Steriade et al., 1993; Steriade & Contreras, 1995). This concept suggested that bursting was not an effective relay mode and that, in the alert state, the relay cells should respond only in tonic mode. However, some bursting has been observed in lightly anesthetized and awake animals, often in response to visual stimuli, suggesting that both response modes may play important roles in signal transmission in the awake state (McCarley et al., 1983; Guido et al., 1992, 1995; Guido & Weyand,1995; Nicolelis et al., 1995; Albrecht et al., 1998; also see reviews by Sherman, 1996; Sherman & Guillery, 1996).

To clarify the state-dependent switching of firing mode, we have recorded extracellularly from thalamic relay cells in unanesthetized monkeys during both sleep and awake, vigilant behavior.

## Methods

Data were collected using single-unit recording techniques in two chronically prepared Rhesus monkeys, treated in accordance with institutional guidelines for animal care. The techniques were adapted from those of Gnadt and Mays (1995). Briefly, recording cylinders and scleral eye coils were surgically positioned on animals. Re-

Address correspondence and reprint requests to: S.M. Sherman, Department of Neurobiology, State University of New York, Stony Brook, NY 11794-5230, USA. E-mail: ssherman@neurobiology.sunysb.edu

cordings were made while the animal sat in a confinement chair with its head fixed. These chairs were comfortable enough for these animals to fall asleep readily during a recording session, which typically lasted for about 2–3 h. The monkeys were trained to fixate a small visual target contained within an error window (a square, 2.5 deg per side). As an inducement to work, their daily intake of water was restricted to that earned as reward for successful completion of fixation trials.

Transdural tungsten electrodes were lowered into the thalamus. Neurons in the lateral geniculate nucleus were identified by their small visual receptive fields, predictable ocular dominance switching as we progressed through the laminae, and a change to larger receptive fields with better contrast sensitivity as we passed from parvocellular (P) to magnocellular (M) laminae. Somatosensory cells were identified by their responsiveness to manually applied somatosensory stimuli and lack of response to either visual or auditory stimuli. When the somatosensory receptive fields were relatively small and strictly contralateral to the recording site, we assumed the cell was in the ventrobasal complex, and when the receptive fields were more diffuse and/or ipsilateral to the recording site or bilateral, we assumed the cell was in the anterior pulvinar (or primate equivalent of the medial portion of the posterior thalamic complex in rodents and carnivores). However, for the purposes of this study, the distinction between these somatosensory thalamic nuclei is irrelevant (see Results).

Individual spikes, monitored on an oscilloscope and isolated through a time/amplitude window discriminator, were recorded by a computer at a sampling time of 0.1ms. Eye position was monitored using the scleral eye coil technique with a resolution of 2 ms temporally and 0.2 deg spatially. Electroencephalographic (EEG) recordings were made to monitor the state of consciousness of the animal by differential recording from cranial bone screws. Since the animal was placed in a magnetic field to record eye movement, it was not possible to record EEG while monitoring fixation. The strength of the oscillating magnetic field overwhelmed the common mode rejection of the EEG amplifier and forced it out of the linear range. By switching between eye movement and EEG recordings we confirmed that aimless, drifting eye movements were associated with slower wave/higher amplitude EEGs. In some experiments where we looked at the effects of a visual stimulus during fixation tasks, the receptive fields of the geniculate units were placed on the screen of a visual display unit (Cambridge Systems VSG 2/3 graphics card). Either the screen displayed a drifting sine-wave grating (for visually driven activity) or displayed a blank field (for spontaneous activity). For most cells, a grating with a spatial frequency of 0.1 cycles/deg, a temporal frequency of 4–5 Hz, and a contrast of 0.9 was sufficient to elicit well-modulated responses.

## Results

Our data set from the two unanesthetized monkeys includes 48 geniculate neurons (17 magnocellular, or M, cells and 31 parvocellular, or P, cells). This includes a subset of 27 geniculate cells (eight M and 19 P) for which we were able to maintain recording through both the sleep and waking states. We studied responses to visual stimulation (drifting, sinusoidal gratings) in the other 21 geniculate cells (nine M and 12 P) during the awake state. We were also able to record from seven cells from the somatosensory thalamus during both sleep and awake states. While we could distinguish between cells in the M and P laminae of the lateral geniculate nucleus and between visual and somatosensory thalamic cells (see Methods), we could not distinguish between relay cells and interneurons, since this requires special techniques (such as antidromic activation from cortex) that were unavailable to us. We assume that most of our recorded cells were relay cells for three reasons: (1) relay cells outnumber interneurons in these nuclei by roughly 3:1; (2) interneurons are smaller and thus less likely to be sampled (Jones, 1985); and (3) interneurons do not normally exhibit bursting based on activation of low-threshold $Ca^{2+}$ spikes (Pape et al., 1994), but all of the cells we recorded showed clear evidence of such bursting (see below).

Spike trains were analyzed and separated into periods of burst and tonic activity. We used two slightly different sets of criteria in our analysis. One we refer to as "conservative," because it practically never identifies a tonic action potential as part of a burst but does misidentify some action potentials in bursts as tonic firing; the other we call "liberal" because it errs on the other side. The rationale for applying both sets of criteria is to obtain upper and lower estimates for the amount of bursting, since with extracellular recording it is impossible to identify bursts more directly. In any case, these criteria affect only quantitative estimates of bursting but not the broader, qualitative conclusions of the study (see below).

For the conservative criteria, we simply adopted those suggested from intracellular recording of geniculate cells in lightly anesthetized cats (Lu et al., 1992). The first action potential in a burst is one showing a preceding silent period of $\geq$100 ms and a following interspike interval of $\leq$4 ms. Any subsequent action potentials with preceding interspike intervals of $\leq$4 ms are additional action potentials in a burst. All other action potentials are considered to represent tonic firing. These criteria are conservative, because, based on the intracellular recording (Lu et al., 1992), $\geq$98% of all action potentials designated as burst mode are actually from bursts riding on a low-threshold $Ca^{2+}$ spike, and tonic firing never shows such an activity pattern. However, roughly 5–10% of action potentials classified as tonic firing may actually be bursts. This is because the first action potential in a burst may occasionally occur after as little as a 50-ms silent period, the second action potential in a burst often has a preceding interspike interval $>$4 ms, and succeeding interspike intervals in a burst grow slightly. Thus, action potentials designated as tonic are much more likely to be mislabeled than are those designated as burst. For our liberal criteria, we reduced the silent period before the first action potential from $\geq$100 ms to $\geq$50 ms, we increased the first interspike interval in the burst to $\leq$6 ms, and we considered all following action potentials as part of the burst if their interspike intervals increased by no more than 2 ms for each succeeding action potential, up to a maximum allowed interspike interval of 16 ms. Finally, a low-threshold $Ca^{2+}$ spike occasionally evokes a single action potential, which by either set of criteria would all be deemed tonic firing, and this special case is considered in the Discussion.

### *Spontaneous activity*

Fig. 1 shows typical firing patterns during spontaneous activity for a geniculate cell. During the alert state (Fig. 1A), evidenced both by a high frequency, low-amplitude EEG plus active fixation by the animal on the visual target, most of the firing seen is in tonic mode, but occasional burst firing is seen (*arrow*). When the animal was asleep, the EEG changed to a discernable slow ($\sim$3 Hz) high-amplitude trace (Fig. 1B), which was coupled with more bursting (*arrows*). However, this was still interspersed with epochs of tonic firing. We noted that, during sleep, cells would appear to switch

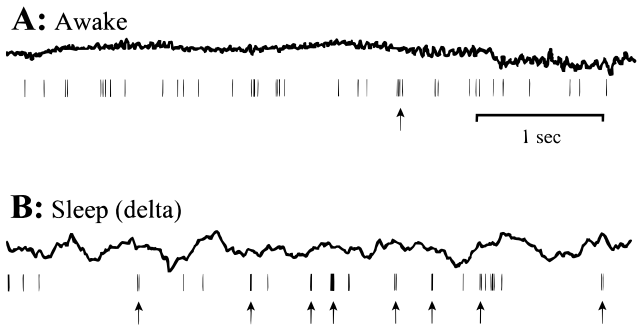


**Fig. 1.** Recordings from a single geniculate neuron during wakefulness and sleep. Each panel has an EEG record above a spike raster, where a vertical line represents each action potential. The arrows depict bursts of action potentials. A: Activity during wakefulness. B: Activity during slow wave (delta) sleep.

intermittently between burst and brief epochs of tonic firing every few hundreds of milliseconds to several seconds.

Fig. 2 summarizes the extent of bursting for these cells, expressed as the percent of all action potentials represented by bursts, in sleep and wakefulness. Fig. 2A shows the results of analyzing the data with our conservative estimate for bursting. Here, the geniculate cells as a group showed more bursting during slow wave sleep than wakefulness. This was true for every one of the eight M cells (mean ± S.D. of 22.5 ± 14.9% during sleep *vs.* 1.6 ± 1.2% during the alert state) and 17 of the 19 P cells (3.6 ± 2.4% during sleep *vs.* 1.2 ± 1.2% during the alert state). These differences for both cell types were significant ($P < 0.001$ on a Wilcoxon matched-pairs test). However, even though both cell types burst more often during slow wave sleep, it was surprising that tonic firing was still more prevalent during this condition. Also, M cells showed significantly more bursting than did P cells during sleep ($P < 0.001$ on a Mann-Whitney *U* Test), but there was no clear difference in the amount of bursting between M and P cells during wakefulness ($P > 0.1$). When the liberal criteria were applied to the geniculate cells (Fig. 2B), we found more bursting, as expected. For P cells, the bursting rate during sleep becomes 8.1 ± 4.1% and 5.3 ± 3.3% during wakefulness, and the difference remains significant ($P < 0.02$). The values for M cells are 30.2 ± 15.5% during sleep and 9.9 ± 5.9% during wakefulness ($P < 0.01$). Thus, even when liberal criteria are used, we estimate that geniculate cell responses during sleep occur mostly in tonic mode.

As shown in Fig. 2, all seven of the somatosensory cells showed more bursting during slow wave sleep (50.8 ± 12.2% or 71.5 ± 11.0% for the conservative or liberal criteria, respectively) than during wakefulness (29.5 ± 16.3% or 44.5 ± 24.8%), and these differences were also significant ($P < 0.02$ for both criteria). Interestingly, during both sleep and wakefulness, the somatosensory cells exhibited more bursting than either class of geniculate cell (on a Mann-Whitney *U* Test, $P < 0.005$ for all comparisons. It is worth emphasizing that even though the greatest ratio of bursting we observed was for somatosensory cells during slow wave sleep, many of the responses of these cells during sleep was in tonic mode, at least according to our criteria.

Correlations of burst levels between sleep and alert states were insignificant (Fig. 2), indicating that a given cell does not show a tendency to be more or less bursty during both sleep and wakefulness.

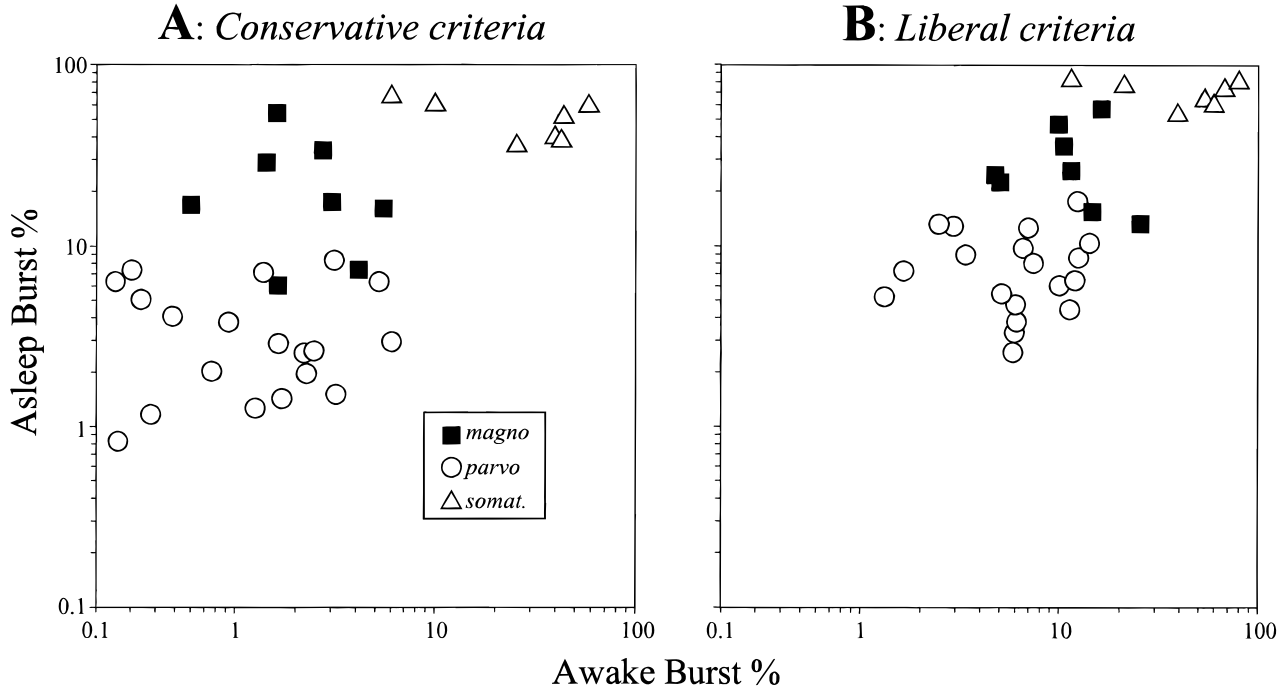


**Fig. 2.** Scatterplots of the percentage of all action potentials occurring within bursts during awake *versus* sleep. Data are shown for all cells for which we obtained recordings during both sleep and waking behavior, including eight M and 19 P cells of the lateral geniculate nucleus and seven thalamic cells with somatosensory receptive fields. A: Scatterplot using the conservative criteria. B: Scatterplot using the liberal criteria.

Fig. 3 shows typical spontaneous firing patterns from a geniculate neuron in sleep and wakefulness. Since EEG recording was precluded by eye position monitoring (see Methods), evidence of alert wakefulness here involved having the monkey actively maintain fixation of a small spot. We analyzed the interspike intervals during spontaneous activity by plotting the interval before each spike against the interval after (Figs. 3A and 3B). When the animal was asleep, the firing of the geniculate cell showed several distinct spike clusters (Fig. 3A). One cluster, seen in the lower right corner, occurred with prespike intervals of ≥100 ms and postspike intervals of ≤4 ms. This represents the first spikes of bursts (Lu et al., 1992). Also, there was a band of spikes, along the left of the graph, with prespike intervals of ≤4 ms and postspike intervals of up to ~300 ms. These are mostly the later spikes of bursts. All other spikes (i.e. the broad middle group) were part of tonic mode activity. Because there were far fewer bursts in this same cell during wakefulness, the burst mode clustering was not as apparent (Fig. 3B).

Recent studies suggested that thalamic firing patterns during sleep should be dominated by bursting that is rhythmic (Steriade & Deschênes, 1984; Domich et al., 1986; Steriade & Llinás, 1988; McCormick & Feeser, 1990; Steriade & McCarley, 1990; Steriade et al., 1990, 1993; Steriade, 1993; McCormick & Bal, 1994, 1997; Contreras & Steriade, 1997). To test for rhythmicity, we constructed autocorrelograms of the firing patterns and submitted these to Fourier analysis. For the geniculate cell illustrated, there was much more bursting during sleep (indicated by the large peak at −4 to +4 ms in Fig. 3C) than wakefulness (Fig. 3D). However, there was no sign of rhythmic activity in either case (Figs. 3C and 3D). This was true for all geniculate cells studied during sleep and/or wakefulness: none showed any significant rhythmic bursting during sleep or wakefulness.

Fig. 4 shows a typical example of spontaneous activity for a cell of the somatosensory thalamus. A markedly different and distinctive four-cluster pattern was seen during slow wave sleep (Fig. 4A). One cluster, at the lower right, has prespike and postspike intervals, respectively, of ≥100 ms and ≤4 ms, which again represent the first spikes in bursts. A second cluster, at the lower left with prespike and postspike intervals ≤4 ms, is the second to penultimate spikes in bursts. The third cluster of spikes, at the upper left, with prespike and postspike intervals of ≤4 ms and ≥100 ms, respectively, is the last spikes in bursts. The fourth cluster, with prespike and postspike intervals of ≥100 ms, is a curious cluster that, by our criteria, is considered tonic firing but may in fact include many burst responses. This is considered further in the Discussion. During wakefulness, there is a subtle change in the clustering (Fig. 4B). The cluster of first and second-to-penultimate spikes in bursts is clear in the lower portions of the graph; but the last spikes in bursts rise as a band from the second-to-penultimate spikes. It is clear from these examples that, during wakefulness, the somatosensory cell exhibited more bursting than did the geniculate cell (Figs. 3A,B and 4A,B). It is particularly interesting that the autocorrelograms for somatosensory thalamus were quite different from the visual cells (Figs. 4C and 4D). For the cell illustrated in Fig. 4 and the other somatosensory cells, Fourier analysis (not shown) of the bursting during sleep showed clear rhythmicity at 3–4 Hz, with no rhythmic bursting during wakefulness.

### *Visually driven activity of geniculate cells in the awake state*

Fig. 5 shows various representations of responses of a geniculate cell from the awake monkey to a grating drifted at 5 Hz; during the stimulation, the monkey fixated a spot as described above. The scatterplots of prespike and postspike intervals (Fig. 5A) show three main clusters. One is a vertical band, on the right, with prespike intervals ≥100 ms. The spikes in this band with postspike intervals of ≤4–6 ms are first spikes in bursts. The spikes in this

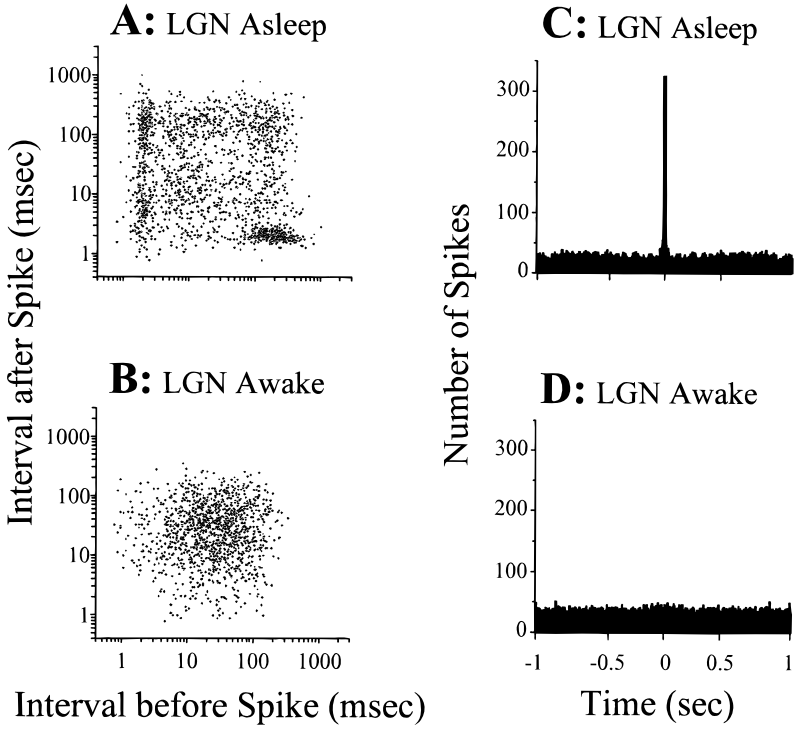


**Fig. 3.** Spontaneous activity of a typical geniculate cell during sleep and wakefulness. A: Scatterplot of interspike intervals during sleep. This plots, for each action potential, the interval before *versus* the interval after. B: Scatterplot of interspike intervals during wakefulness. C: Autocorrelogram for the responses during sleep. D: Autocorrelogram for the responses during wakefulness. Autocorrelograms do not include the 0 time bin, and this applies also to Figs. 4C and 4D.

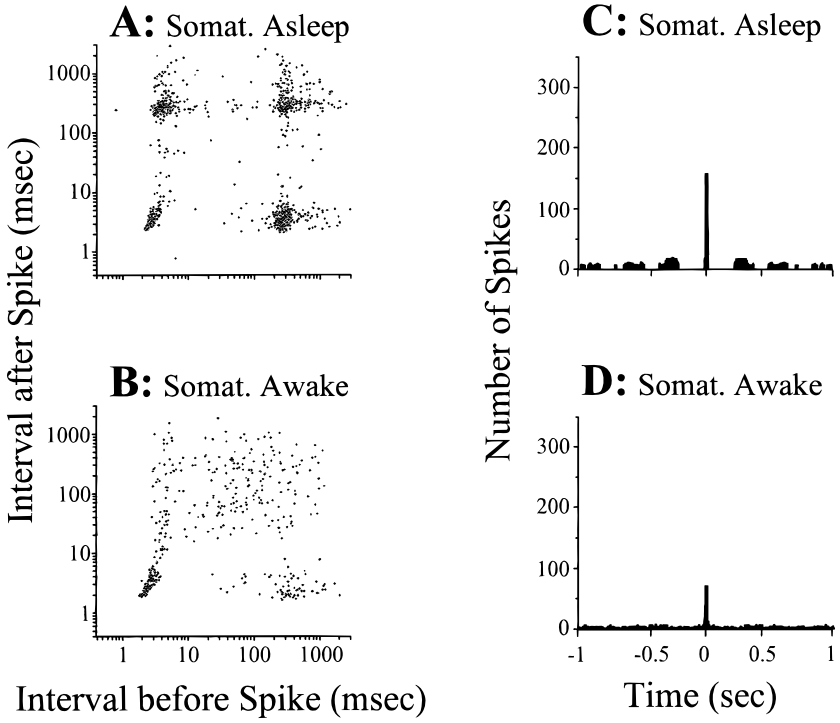


**Fig. 4.** Spontaneous activity of a typical cell of somatosensory thalamus during sleep and wakefulness; conventions as in Fig. 3. A: Scatterplot of interspike intervals during sleep. B: Scatterplot of interspike intervals during wakefulness. C: Autocorrelogram for the responses during sleep. D: Autocorrelogram for the responses during wakefulness.

band with longer postspike intervals are difficult to identify in terms of firing mode and are considered further in the Discussion. A second cluster is a horizontal band, at the top, with postspike intervals of ≥100 ms, and these include the last spikes before a burst. The third cluster, in the middle, is the rest of the spikes, most of which represent tonic firing. This pattern of clustering was not obvious during spontaneous activity of the same cell during equivalent periods of wakefulness with fixation of the spot (Fig. 3B), and thus it may be that strong stimulation is necessary to reveal these clusters. The autocorrelogram of Fig. 5B shows that the only obvious rhythmic firing is related to the temporal cycle of the drifting grating. Fourier analysis (not shown) revealed a single peak at the stimulus frequency. We found this true for all geniculate cells during visual stimulation: the autocorrelograms revealed rhythmicity related only to the temporal frequency of the visual stimulus. Fig. 5C shows an interspike interval histogram for the same cell in Figs. 5A and 5B. The open arrow shows a small, secondary peak after intervals of ≥100 ms which reflects the vertical cluster with prespike intervals of ≥100 ms in Fig. 5A.

We observed this same general pattern of response in every one of the 21 geniculate cells (nine M and 12 P) for which we used drifting sinusoidal gratings as the visual stimulus (see Methods for the grating variables). Further examples for three other cells are shown in Fig. 6. Using the conservative criteria, we estimated that the relative number of action potentials in bursts to these visual stimuli were 2.9 ± 2.6% for M cells and 1.0 ± 1.3 for P cells ($P > 0.05$ on a Mann-Whitney *U* Test). With our liberal criteria, these values rose to 10.3 ± 10.3 for M cells and 5.9 ± 4.6 for P cells ($P > 0.1$).

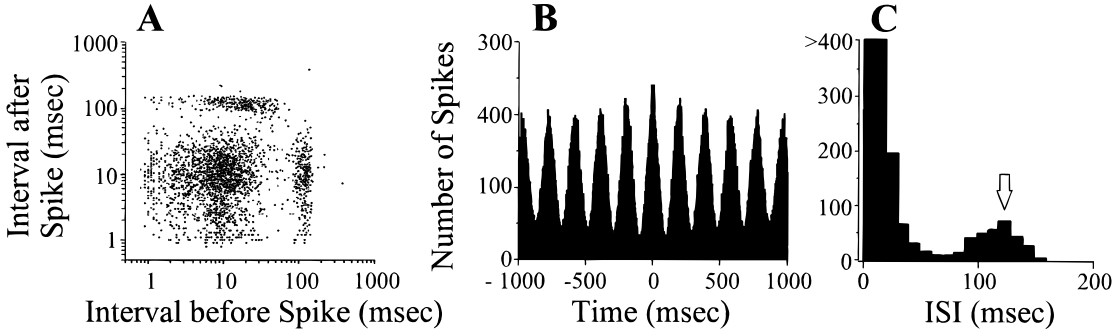


**Fig. 5.** Patterns of spike discharge of representative geniculate cell in response to a sine-wave grating drifted at 5 Hz. A: Scatterplot of interspike intervals as in Fig. 3A. B: Autocorrelation of responses. C: Interspike interval histogram. The open arrow in C points to a later peak of intervals ≥100 ms that also reflects the vertical cluster in A.

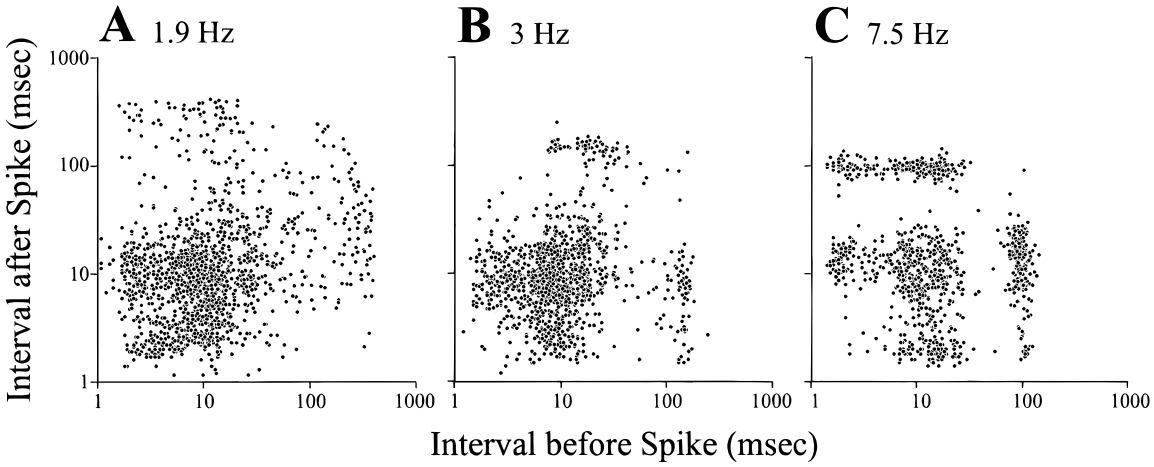


**Fig. 6.** Patterns of spike discharge of three other representative geniculate cells in response to a sine-wave grating as in Fig. 5A. A: Responses to grating drifted at 1.9 Hz. B: Responses to grating drifted at 3 Hz. C: Responses to grating drifted at 7.5 Hz.

## Discussion

In light of what is now known about the low-threshold calcium current (Jahnsen & Llinàs, 1984*a*,*b*; Steriade & Llinàs, 1988), our results lead to several novel conclusions that support much of the findings in the unanesthetized cat (McCarley et al., 1983; Guido & Weyand, 1995). First, it is clear that both firing modes—tonic and burst—can be recognized in thalamic cells of unanesthetized monkeys, both during awake vigilant behavior and sleep. Second, for geniculate cells, tonic firing predominated under *both* conditions, although more bursting was seen during sleep. However, we saw no evidence of rhythmic bursting of these visual cells in any condition. Third, for somatosensory cells, we saw a significantly higher amount of bursting than that in geniculate cells both during sleep and wakefulness. Unlike the geniculate cells, the somatosensory neurons showed rhythmic bursting during sleep. Moreover, these observations suggest a fourth conclusion: that there may be fundamental differences in how cells of different thalamic nuclei express these firing modes.

### *Differences between thalamic nuclei*

On average, geniculate neurons exhibit less burst mode activity than somatosensory cells. In the awake state, one could argue that the visual fixation task creates a difference between the visual and somatosensory relays, resulting in more tonic firing for the former. However, this cannot explain the difference seen during sleep, when the somatosensory neurons continue to display a high level of *rhythmic* bursting compared to the lower level of *arrhythmic* bursting seen in geniculate cells. This refutes the current dogma that rhythmic bursting is a ubiquitous feature of relay cells throughout thalamus during sleep (Steriade et al., 1993). Most earlier recordings that supported this conclusion were made not in naturally sleeping animals but in anesthetized subjects, and perhaps the anesthesia is more conducive to the dominance of rhythmic bursting.

However, we did observe pronounced rhythmic bursting in our sample of somatosensory cells, and it is possible that the lateral geniculate nucleus is an atypical thalamic nucleus in this regard. It may be of particular interest that an earlier study in the unanesthetized cat reported that only five of 26 neurons in the lateral geniculate nucleus showed rhythmic bursting during slow wave sleep (McCarley et al., 1983). In contrast, neurons of the VA/VL thalamic complex reported in a different study of unanesthetized cats exhibited mainly rhythmic bursting during slow wave sleep (Domich et al., 1986). One might have regarded the difference between these earlier studies as reflecting subtle differences between laboratory methodologies. However, our results, from the same animals and often during the same recording sessions, indicate a real difference between thalamic nuclei.

Perhaps geniculate cells fire more tonically and less rhythmically due to the spontaneous activity of their retinal afferents, which might keep them in a relatively more depolarized state with more inactivation of $I_T$ compared to other thalamic cells. For instance, there is evidence that rhythmicity (if not bursting) of geniculate cells in anesthetized cats is greatly enhanced when the retinal inputs are silenced (Nunez et al., 1992), which certainly implicates the effect of sustained background firing of retinal inputs on the firing behavior of their target geniculate cells. This background activity would create more depolarized geniculate cells with little or no de-inactivated $I_T$, resulting in fewer or smaller low-threshold $Ca^{2+}$ spikes. Perhaps other thalamic nuclei (e.g. somatosensory relays) lack such a sustained and depolarizing background influence and do indeed show more rhythmic bursting during sleep.

### *Extent of bursting in thalamic cells*

Direct confirmation that burst firing in unanesthetized monkeys is a result of low-threshold $Ca^{2+}$ is not practical in these behavioral experiments. However, $I_T$ has been documented for every relay cell of every thalamic nucleus of every mammalian species so far studied, including cats, ferrets, guinea pigs, and rats (for details, see Jahnsen & Llinás, 1984*a*,*b*; Deschênes et al., 1984; Crunelli et al., 1989; McCormick & Feeser, 1990; Scharfman et al., 1990; Lo et al., 1991; Bal et al., 1995; reviewed in Sherman & Guillery, 1996), and we have also seen this in *in vitro* preparations of the monkey thalamus (our unpublished observations). It has also been indirectly identified in human (Tsoukatos et al., 1997; Lenz et al., 1998) and monkey (Dougherty et al., 1997) thalamus using techniques similar to those we have used. Clearly, the criteria we have used reveal a particular pattern of action potentials, with interspike intervals of $\leq$4–6 ms (or slightly longer) following a lengthy

silent period (≥50–100 ms), and these are more prevalent during sleep than wakefulness (see Figs. 3 and 4). The silent period is consistent with the period of hyperpolarization needed to de-inactivate $I_T$ so that the $Ca^{2+}$ spike can be activated.

As noted in the Results, we have used both conservative and liberal criteria for bursting to obtain a reasonable range of estimates for bursting. We see variations across cell types (i.e. geniculate M *vs.* P and visual *vs.* somatosensory thalamus). Nonetheless, we conclude that geniculate cells in the awake, behaving monkeys of our study have 5–10% of their action potentials in bursts, and for somatosensory thalamic cells, the value is 30–45%. But even these values may be underestimates for bursting.

One particularly interesting group of action potentials that we have identified by both sets of criteria as tonic firing may in fact represent burst firing. This is the vertical band of action potentials, to the right, seen in the scatterplot of Fig. 5A occurring with a prespike interval of >100 ms and also appearing as a small, secondary peak >100 ms in the interspike interval histogram of Fig. 5C. There are two plausible explanations for this pattern of firing. One is that those spikes in this group with postspike intervals of >4–6 ms really is mostly tonic firing, and that it reflects the fact that the stimuli we use alternately excite and inhibit the cell as each cycle of the sinusoidal grating passes over the receptive field. Each excitatory half-cycle follows an inhibitory one during which the cell would be hyperpolarized and silent, creating a gap in firing and leading to the secondary peak in the interspike interval histogram. One might expect that the latency of this secondary peak would rapidly decrease with increasing temporal frequency, but the actual relationship between this secondary peak and temporal frequency is probably a more complex function that also involves the temporal-frequency tuning of excitation and inhibition.

Another plausible explanation is that this small, secondary peak in the interspike interval histogram instead represents burst firing. Sufficient hyperpolarization for 100 ms or so, presumably reflecting the preceding silent period noted above, will de-inactivate $I_T$. The extent of this $I_T$ de-inactivation is monotonically related to the amplitude (up to about −80 mV) and duration (up to about 100 ms) of the hyperpolarization (Coulter et al., 1989). Consequent depolarization activates $I_T$, which produces the low-threshold $Ca^{2+}$ spike. This $Ca^{2+}$ spike is effectively activated in an all-or-none fashion, but its size depends on the extent of preceding $I_T$ de-inactivation (Zhan et al., 1999). De-inactivation may thus be incomplete if the hyperpolarization is too small or too short, and this will produce a smaller low-threshold $Ca^{2+}$ spike. Nonetheless, most $Ca^{2+}$ spikes are sufficiently large to activate bursts of ≥2 action potentials. However, it is possible to activate a smaller $Ca^{2+}$ spike due to less complete $I_T$ de-inactivation such that a single action potential may result. We have seen this in our intracellular recordings of rat, cat, and monkey thalamic cells (unpublished observations). This suggests that in the driven state many action potentials do not fit our burst criteria because, although they are preceded by a silent period of ≥100 ms, the next action potentials occur later than 4 ms and thus they may be single spikes in burst mode (the vertical cluster in Fig. 5A). If this explanation is correct, namely, that most action potentials following a ≥100 ms silent period are associated with $Ca^{2+}$ spikes, then such a response may be considered an extreme example of a burst response. That is, the burst has only a single action potential. A "single-spike burst" may seem an oxymoron, but the point is that this pattern of action potentials evoked by $Ca^{2+}$ spikes may have a very different significance than the single spikes seen during tonic firing especially in visually active animal.

Whether this pattern of firing leading to the secondary peak of action potentials in the interspike interval histograms reflects tonic firing, single-spike bursts, or a combination, is not possible to discern from our data. However, there are two additional reasons to consider seriously the possibility that this reflects burst firing. First, the fact that these action potentials follow a long period of silence suggests that the relay cell may be actively inhibited and thus hyperpolarized during this period. Such hyperpolarization would be expected to de-inactivate $I_T$, which in turn means that the firing that does immediately follow is very likely to be relayed to activation of $I_T$ and low-threshold $Ca^{2+}$ spikes. Second, the pattern of firing seen in somatosensory cells during sleep is also best explained by assuming the presence of many single-spike bursts. As noted in the Results, the scatterplots of interspike intervals for this cell show four clusters (Fig. 4A), and while three of them seem clearly related to bursts (i.e. the clusters with prespike and postspike intervals, respectively, of ≥100 ms and ≤4 ms, ≤4 ms and ≤4 ms, and ≤4 ms and ≥100 ms), the fourth, with prespike and postspike intervals of ≥100 ms and 100 ms, does not. We have termed this tonic firing, but perhaps an equally plausible explanation for this is that it represents single-spike bursts.

If so, then we have underestimated bursting in these thalamic cells. For instance, we would now conclude that nearly all spikes from somatosensory cells in the sleeping animal reflect burst firing, instead of the value of roughly 50–70% reported in the Results, and this is more in line with expectations from the literature suggesting that nearly all firing of these thalamic cells during slow wave sleep is rhythmic and in burst mode (Steriade et al., 1993). Yet even if we use these less stringent criteria that allow for single-spike bursts, we would still fail to see predominantly rhythmic bursting for geniculate cells during sleep. However, this possibility of single-spike bursts does imply that we have substantially underestimated the extent of bursting in the waking animal, especially for geniculate cells responding to visual stimuli. Here, with our conservative criteria, our estimates for burst responses to visual stimulation for geniculate cells now changes from a mean ± S.D. of 2.9 ± 2.5% to 15.1 ± 3.7% for M cells and from 1.0 ± 1.3% to 10.2 ± 6.9% for P cells. With liberal criteria, the values are 22.5 ± 3.9% for M cells and 14.9 ± 7.3% for P cells.

### *Role of burst firing in the awake state*

Regardless of which value is correct, our results indicate that the burst firing mode is not strictly limited to sleep but can occur during awake, vigilant behavior. This basic observation has also been made from studies of the lateral geniculate nucleus in awake cats (McCarley et al., 1983; Guido & Weyand, 1995). Although the percentage of action potentials we observed during burst firing for geniculate cells in the behaving monkeys was somewhat low, there are at least two reasons to think it might be significant for visual processing. First, one suggestion for burst firing is that it serves as a sort of "wake up call" for novel but previously unattended stimuli (Sherman, 1996), and as such the amount of bursting needed to occasionally redirect attention might be quite low. Since our visual stimuli were on constantly, there was perhaps minimum novel stimulation, and our experimental design may thus have biased against seeing more bursting. Perhaps the relative ratio of burst to tonic firing can vary considerably under different behavioral regimens and be considerably higher for certain requirements of sensory processing than seen here. Second, the visual stimuli employed had no particular meaning to the animals, who in this case were not required to perform visual tasks, such as detecting novel or chang-

ing stimuli, that has been suggested as a possible inducement to burst firing (Sherman, 1996). Certainly the occurrence of the temporally unique pattern of single spike bursts should be given more consideration as being functionally significant in this respect. Because low spontaneous activity is associated with burst mode, a "single spike" burst may indeed play a significant role as a "wake up call." Related to this, many synapses in cortex show the phenomenon of depression (cf. Abbott et al., 1997; Varela et al., 1999), suggesting that the evoked excitatory postsynaptic potential (EPSP) in cortex from geniculocortical axons would be considerably greater for a single action potential following a long silent period (which would reduce or eliminate the suppression) than after shorter intervals (which would promote depression). A larger EPSP thus might be an excellent candidate for a "wake up call."

## References


Abbott, L.F., Varela, J.A., Sen, K. & Nelson, S.B. (1997). Synaptic depression and cortical gain control. *Science* **275**, 220–224.

Albrecht, D., Royl, G. & Kaneoke, Y. (1998). Very slow oscillatory activities in lateral geniculate neurons of freely moving and anesthetized rats. *Neuroscience Research* **32**, 209–220.

Bal, T., Von Krosigk, M. & McCormick, D.A. (1995). Synaptic and membrane mechanisms underlying synchronized oscillations in the ferret lateral geniculate nucleus *in vitro*. *Journal of Physiology* (London) **483**, 641–663.

Contreras, D. & Steriade, M. (1997). State-dependent fluctuations of low-frequency rhythms in corticothalamic networks. *Neuroscience* **76**, 25–38.

Coulter, D.A., Huguenard, J.R. & Prince, D.A. (1989). Calcium currents in rat thalamocortical relay neurons: Kinetic properties of the transient, low threshold current. *Journal of Physiology* (London) **414**, 587–604.

Crunelli, V., Lightowler, S. & Pollard ,C.E. (1989). A T-type $Ca^{2+}$ current underlies low-threshold $Ca^{2+}$ potentials in cells of the cat and rat lateral geniculate nucleus. *Journal of Physiology* (London) **413**, 543–561.

Deschênes, M., Paradis, M., Roy, J.P. & Steriade, M. (1984). Electrophysiology of neurons of lateral thalamic nuclei in cat: Resting properties and burst discharges. *Journal of Neurophysiology* **51**, 1196–1219.

Domich, L., Oakson, G. & Steriade, M. (1986). Thalamic burst patterns in the naturally sleeping cat: A comparison between cortically projecting and reticularis neurones. *Journal of Physiology* (London) **379**, 429–449.

Dougherty, P.M., Li, Y.J., Lenz, F.A., Rowland, L. & Mittman, S. (1997). Correlation of effects of general anesthetics on somatosensory neurons in the primate thalamus and cortical EEG power. *Journal of Neurophysiology* **77**, 1375–1392.

Gnadt, J.W. & Mays, L.E. (1995). Neurons in monkey parietal area LIP are tuned to for eye movement parameters in three-dimensional space. *Journal of Neurophysiology* **73**, 280–297.

Guido, W., Lu, S.-M. & Sherman, S.M. (1992). Relative contributions of burst and tonic responses to the receptive field properties of lateral geniculate neurons in the cat. *Journal of Neurophysiology* **68**, 2199–2211.

Guido, W., Lu, S.-M., Vaughan, J.W., Godwin, D.W. & Sherman, S.M. (1995). Receiver operating characteristics (ROC) analysis of neurons in the cat's lateral geniculate nucleus during tonic and burst response modes. *Visual Neuroscience* **12**, 723–741.

Guido, W. & Weyand , T. (1995). Burst responses in thalamic relay cells of the awake behaving cat. *Journal of Neurophysiology* **74**, 1782–1786.

Jahnsen, H. & Llinàs, R. (1984*a*). Electrophysiological properties of guinea-pig thalamic neurones: An *in vitro* study. *Journal of Physiology* (London) **349**, 205–226.

Jahnsen, H. & Llinàs, R. (1984*b*). Ionic basis for the electroresponsive and oscillatory properties of guinea-pig thalamic neurones *in vitro*. *Journal of Physiology* (London) **349**, 227–247.

Jones, E.G. (1985). *The Thalamus*. New York: Plenum Press.

Lenz, F.A., Garonzik, I.M., Zirh, T.A. & Dougherty, P.M. (1998). Neuronal activity in the region of the thalamic principal sensory nucleus (ventralis caudalis) in patients with pain following amputations. *Neuroscience* **86**, 1065–1081.

Livingstone, M.S. & Hubel, D. (1981). Effects of sleep and arousal on the processing of visual information in the cat. *Nature* (London), **291**, 554–561.

Lo, F.-S., Lu, S.-M. & Sherman, S.M. (1991). Intracellular and extracellular *in vivo* recording of different response modes for relay cells of the cat's lateral geniculate nucleus. *Experimental Brain Research* **83**, 317–328.

Lu, S.-M., Guido, W. & Sherman, S.M. (1992). Effects of membrane voltage on receptive field properties of lateral geniculate neurons in the cat. *Journal of Neurophysiology* **68**, 2185–2198.

McCarley, R.W., Benoit, O. & Barrionuevo, G. (1983). Lateral geniculate nucleus unitary discharge in sleep and waking: State- and rate-specific aspects. *Journal of Neurophysiology* **50**, 798–818.

McCormick, D.A. & Bal, T. (1994). Sensory gating mechanisms of the thalamus. *Current Opinion in Neurobiology* **4**, 550–556.

McCormick, D.A. & Bal, T. (1997). Sleep and arousal: Thalamocortical mechanisms. *Annual Review of Neuroscience* **20**, 185–215.

McCormick, D.A. & Feeser, H.R. (1990). Functional implications of burst firing and single spike activity in lateral geniculate relay neurons. *Neuroscience* **39**, 103–113.

Nicolelis, M.A., Baccala, L.A., Lin, R.C. & Chapin, J.K. (1995). Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. *Science* **268**, 1353–1358.

Nunez, A., Amzica, F. & Steriade, M. (1992). Intrinsic and synaptically generated delta (1–4 Hz) rhythms in dorsal lateral geniculate neurons and their modulation by light-induced fast (30–70 Hz) events. *Neuroscience* **51**, 269–284.

Pape, H.-C., Budde, T., Mager, R. & Kisvárday, Z.F. (1994). Prevention of $Ca^{2+}$-mediated action potentials in GABAergic local circuit neurones of rat thalamus by a transient $K^+$ current. *Journal of Physiology* (London) **478**, 403–422.

Scharfman, H.E., Lu, S.-M., Guido, W., Adams, P.R. & Sherman, S.M. (1990). N-methyl-D-aspartate (NMDA) receptors contribute to excitatory postsynaptic potentials of cat lateral geniculate neurons recorded in thalamic slices. *Proceedings of the National Academy of Sciences of the U.S.A.* **87**, 4548–4552.

Sherman, S.M. (1996). Dual response modes in lateral geniculate neurons: Mechanisms and functions. *Visual Neuroscience* **13**, 205–213.

Sherman, S.M. & Guillery, R.W. (1996). Functional organization of thalamocortical relays. *Journal of Neurophysiology* **76**, 1367–1395.

Steriade, M. (1993). Central core modulation of spontaneous oscillations and sensory transmission in thalamocortical systems. *Current Opinion in Neurobiology* **3**, 619–625.

Steriade, M. & Contreras, D. (1995). Relations between cortical and thalamic cellular events, during transition from sleep patterns to paroxysmal activity. *Journal of Neuroscience* **15**, 623–642.

Steriade, M. & Deschênes, M. (1984). The thalamus as a neuronal oscillator. *Brain Research Reviews* **8**, 1–63.

Steriade, M., Jones, E.G. & Llinás, R. (1990). *Thalamic Oscillations and Signalling*. New York: Wiley.

Steriade, M. & Llinàs, R. (1988). The functional states of the thalamus and the associated neuronal interplay. *Physiological Reviews* **68**, 649–742.

Steriade, M. & McCarley, R.W. (1990). *Brainstem Control of Wakefulness and Sleep*. New York:Plenum Press.

Steriade, M., McCormick, D.A. & Sejnowski, T.A. (1993). Thalamocortical oscillations in the sleeping and aroused brain. *Science* **262**, 679.

Tsoukatos, J., Kiss, Z.H.T., Davis, K.D., Tasker, R.R. & Dostrovsky, J.O (1997). Patterns of neuronal firing in the human lateral thalamus during sleep and wakefulness. *Experimental Brain Research* **113**, 273–282.

Varela, J.A., Song, S., Turrigiano, G.G. & Nelson, S.B. (1999). Differential depression at excitatory and inhibitory synapses in visual cortex. *Journal of Neuroscience* **19**, 4293–4304.

Zhan, X.J., Cox, C.L., Rinzel, J. & Sherman, S.M. (1999). Current clamp and modeling studies of low threshold calcium spikes in cells of the cat's lateral geniculate nucleus. *Journal of Neurophysiology* **81**, 2360–2373.