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Higher-order thalamic relays burst more than first-order relays

E. J. Ramcharan*, J. W. Gnadt†, and S. M. Sherman‡§

*Center for Complex Systems and Brain Sciences, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431; †Department of Neurobiology, State University of New York, Stony Brook, NY 11794-5230; and ‡Department of Neurobiology, Pharmacology, and Physiology, University of Chicago, MC 0926, 316 Abbott, 947 East 58th Street, Chicago, IL 60637

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There is a strong correlation between the behavior of an animal and the firing mode (burst or tonic) of thalamic relay neurons. Certain differences between first- and higher-order thalamic relays (which relay peripheral information to the cortex versus information from one cortical area to another, respectively) suggest that more bursting might occur in the higher-order relays. Accordingly, we recorded bursting behavior in single cells from awake, behaving rhesus monkeys in first-order (the lateral geniculate nucleus, the ventral posterior nucleus, and the ventral portion of the medial geniculate nucleus) and higher-order (pulvinar and the medial dorsal nucleus) thalamic relays. We found that the extent of bursting was dramatically greater in the higher-order than in the first-order relays, and this was correlated with lower spontaneous activity in the higher-order relays. If bursting effectively signals the introduction of new information to a cortical area, as suggested, this may be more important in corticocortical transmission than in transmission of primary information to cortex.

thalamus

A key feature of the thalamus is the ability of its relay cells to fire in two distinct modes (called tonic and burst), and the firing mode is determined by the inactivation state of voltage-gated, T-type Ca^{2+} channels in the membranes of the soma and dendrites (1–3). This firing mode strongly affects the nature of the signal that is relayed to the cortex (4). For example, compared with tonic mode, burst mode produces much more nonlinear distortion in the relay of information, but the information relayed has greater detectability because of a greater signal-to-noise ratio and stronger activation of postsynaptic cortical targets (4–9). From these properties, the hypothesis has been forwarded that burst firing, with its greater detectability and cortical activation, serves as a "wake-up call" to the cortex that there has been a change in the outside world (e.g., a novel stimulus within the receptive field of a relay cell for one of the sensory thalamic nuclei); tonic mode, with its more linear relay of information, is then suited better for a more faithful analysis of the relayed information (4, 7). Thus, burst mode would be the most effective for cells dealing with information that is not fully attended to, and the burst would help to redirect attention to the novel stimulus and, ultimately, lead to a shift in firing to tonic mode.

Evidence of burst and tonic firing has been reported in various species during waking behavior, including cats (10–12), rats (13–16), guinea pigs (17, 18), rabbits (5, 6), monkeys (19), and humans (20–22). In general, bursting is relatively rare during full wakefulness and more common during periods of inattention or drowsiness (6). However, nearly all of these data were obtained from cells in first-order thalamic relays, namely, the lateral geniculate nucleus, ventral portion of the medial geniculate nucleus, and the ventral posterior nucleus. These are called "first order," because they represent the first relay of a particular sort of information to the cortex. They are contrasted with the higher-order relays, such as the pulvinar for vision, the magnocellular (or "nonlemniscal") portion of the medial geniculate nucleus for hearing, and the posterior medial nucleus for somesthesis, which are thought to serve as a link in cortico-thalamo-cortical pathways that continue to process these information streams.

We thought that it would be useful to extend the observations of bursting to higher-order thalamic relays in the behaving monkey for the following reasons. Firing mode is largely controlled by various inputs to relay cells that affect their membrane potential and, thus, the inactivation state of the T-type Ca^{2+} channels. There are at least two main differences in the innervation patterns between first- and higher-order thalamic relays that may be relevant here; first, cholinergic inputs from the midbrain parabrachial region cause hyperpolarization of relay cells more frequently in higher-order than in first-order relays (23, 24); and second, a GABAergic input from the zona incerta facilitates selectively targets higher-order thalamic relays (25). This suggests extra hyperpolarization of relay cells in higher-order thalamic relays, which in turn suggests the possibility of more bursting among these relay cells. We sought to test this possibility.

Methods

The experiments were performed on two macaque monkeys (one *Macaca radiata* and *Macaca mulatta*), adhering to strict guidelines of the Institutional Animal Care and Use Committee (●●●). They were housed in a 12-h dark/12-h light cycle, and their water intake was restricted to what was earned as reward for correct completion of tasks. Details are given in E.J.R. *et al.* (26).

Briefly, we made single-unit recordings in various thalamic nuclei of monkeys that had recording chambers, restraining head posts, and scleral eye coils (to record eye position) surgically implanted. During recording sessions, the animals were placed in a restraining chair that was customized for a single animal usage to optimize the comfort level of the subject. All recordings were made while the heads of the animals were fixed.

Tungsten electrodes were advanced by means of a hydraulic microdrive into the nucleus of interest. These monkeys were used initially in studies of the lateral geniculate nucleus, and the borders of this nucleus had been plotted previously. This was simply done by recording the transitions in ocular dominance of visual responses (representing the ocular dominance of the six layers of the lateral geniculate nucleus). All of the other nuclei were located relative to the lateral geniculate nucleus, and when possible, sites were confirmed by using sensory stimuli for the functional modality of the nucleus. Recordings were made in three first-order nuclei (the lateral geniculate nucleus, the ventral portion of the medial geniculate nucleus, and the ventral posterior nucleus; data from lateral and medial portions were combined) and in two higher-order nuclei (the pulvinar and the

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Abbreviation: M*n*, muscarinic receptor *n*.

§To whom correspondence should be addressed. E-mail: msherman@bsd.uchicago.edu.

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medial dorsal nucleus). We could not use responses to sensory stimuli to confirm recordings in the medial dorsal nucleus, and instead, we relied on its large size, so that by using triangulation aimed at its center from the known position of the lateral geniculate nucleus, we were confident that our recordings that were aimed there included medial dorsal cells.

In addition to comparing different thalamic nuclei, we compared two different behavioral conditions. First was the "fixation condition," in which monkeys were trained to fixate a small target (≈0.5°) that was displayed on a monitor located ≈60 cm in front of the animal. The viewable surface of the monitor subtended an area of 40° × 30°. A trial began when the target was displayed, and after a maximum acquisition time of 300 ms, the animal had to hold fixation for ≥3 s, at which time the trial ended and a drop of juice was dispensed as reward. Data for this condition were taken from the time of acquisition of the target to the end of the trial. Recordings were also made of each neuron while the animal was idle (i.e., the "idle condition"); that is, the animal was not made to perform a fixation task and simply sat in the chair. Because the fixation state requires active fixation, we can be reasonably confident that this ●● olves a state of alert vigilance. The idle state is harder to define behaviorally but is likely to involve a lower level of vigilance. In any case, the responses of neurons during the two conditions were remarkably similar (see *Results*).

Results

We recorded from a total of 65 thalamic relay neurons. These ●● luded 40 from first-order thalamic relays (7 from the ventral portion of the medial geniculate nucleus, 24 from the ventral posterior nucleus, and 9 from the lateral geniculate nucleus), and 25 from higher-order thalamic relays (15 from pulvinar and 10 from the medial dorsal nucleus). For these extracellular recordings, we used the criteria based on the intracellular study of Lu *et al.* (27) to classify action potentials as either in burst or tonic mode. Any action potential with a preceding interval of ≥100 ms, followed by action potentials within 4 ms of each other, were all counted as burst mode. We had shown earlier that the basic cellular mechanisms that apply to burst firing for thalamic neurons are also present in the monkey (28). All other action potentials that did not fit into this category were counted as tonic mode. Although we did occasionally evoke sensory responses to help localize recording sites, the data shown below represent spontaneous activity, which we operationally define here as responses that were not elicited by any known sensory or similar stimuli.

Attribution of the first-order relay cells in our sample to their appropriate nuclei was fairly straightforward. We started by plotting the borders of the lateral geniculate nucleus, so relay cells there were easily determined, and initial localization of other thalamic cells was based on the coordinates described in ref. 29. We confirmed recording cells of the ventral posterior nucleus by the receptive fields of these cells, which were small and localized to the contralateral face or limbs. Confirmation of pulvinar recording sites was based both on responses (often weak, but clear) to visual stimuli (drifting sine-wave gratings) as well as their position relative to the lateral geniculate nucleus. Although we did not attempt to drive cells of the ventral portion of the medial geniculate nucleus with a defined auditory stimulus, we were able to excite these neurons effectively and reliably with simple, everyday sounds, like the jangling of a key or tapping on a bench. Cells of the ventral posterior and medial geniculate nuclei never responded to visual stimulation.

Fig. 1 shows data during the fixation condition (see *Methods*) for neurons from first-order thalamic relays (the lateral geniculate nucleus, the ventral posterior nucleus, and the ventral portion of the medial geniculate nucleus). (Because of the similarity of responses during the idle condition, we do not show individual examples here, but summary data for the two conditions are shown below.) The interspike interval plots (*Upper*) show that the vast majority of the points representing action potentials fell outside the criteria for action potentials in burst mode. That is, by the criteria established for the beginning of a

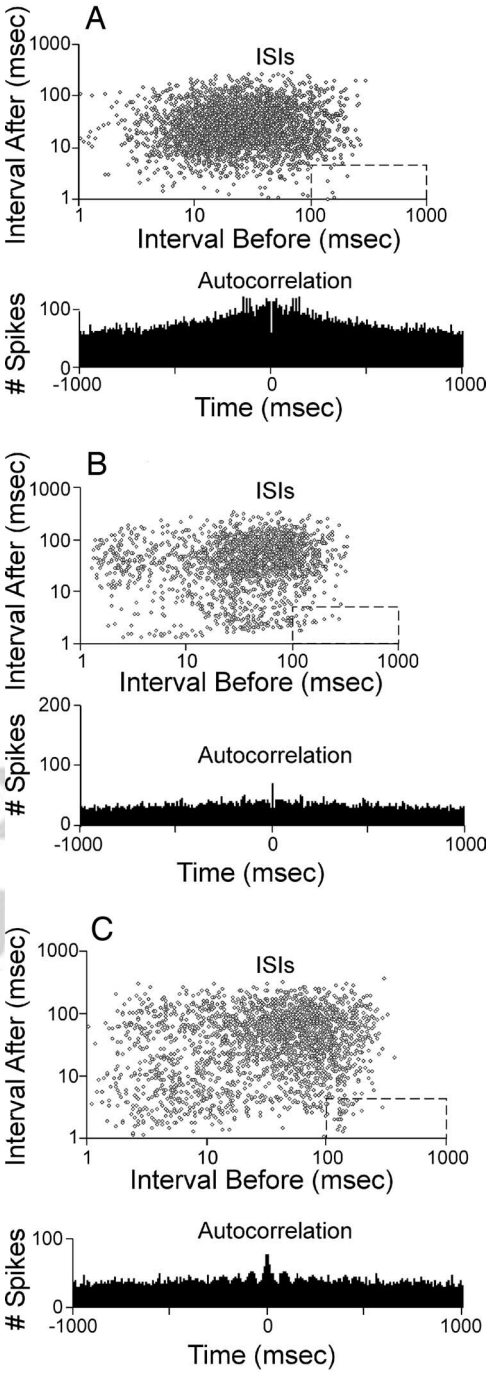

Fig. 1. Responses of neurons from first-order thalamic relays. *Upper* image for each neuron represents a two-dimensional interspike interval ● for which the abscissa indicates the interval to the previous action potential and the ordinate indicates the interval to the next one. The points within the dashed rectangles at the lower right of each plot represent the first action potentials of bursts (see text for details). *Lower* images show autocorrelograms for the same neurons. (*A*) Examples from a neuron of the lateral geniculate nucleus. (*B*) Examples from a neuron of the ventral posterior nucleus. (*C*) Examples from ● uron of the ventral portion of the medial geniculate nucleus. ISIs, ●● AQ: P AQ: Q

F1

burst (outlined by the dashed rectangle in these and similar figures), there were few bursts, the majority of action potentials occurring during the tonic firing mode. (Note that we use a conservative set of criteria, so that virtually no action potentials designated as parts of bursts are misidentified, but more action potentials designated as tonic firing are actually in bursts; for details of the criteria, see refs. 19 and 27.) Our measure for burstiness is the percentage of burst mode firing, which we define as the percentage of all action potential during a response period in question that occur during bursts. For the population of first-order relay neurons, these burst percentages were 0.4 ± 0.1 (mean ± SEM) for the lateral geniculate nucleus ($n = 9$), 2.7 ± 0.7 for the ventral posterior nucleus ($n = 24$), and 3.8 ± 2.5 for the ventral portion of the medial geniculate nucleus ($n = 7$). This means that >90% of the activity for these first-order relay cells was in tonic mode when the animal was performing the visual-fixation task. Strangely, cells of the lateral geniculate nucleus showed the least bursting, and levels here were lower than the levels found in the other first-order relay cells ($P < 0.02$, Wilcoxon

AQ: H

We also used the action-potential-interval data to plot autocorrelograms to test for rhythmic firing. Fig. 1 *A–C Lower* shows there was little evidence of rhythmicity while the animal was actively fixating.

Unlike the findings presented above for the first-order nuclei, the responses of relay neurons of the higher-order relays (i.e., the pulvinar and medial dorsal nucleus) were strikingly different, and again, we saw no difference between the fixation and idle conditions for these neurons. This is shown in Fig. 2 for a pulvinar neuron and two neurons of the medial dorsal nucleus. In the interspike interval plots of each of these neurons (Fig. 2 *A–C Upper*), there are four distinct clusters. The cluster in the bottom right corner represents mostly the first action potentials of bursts (because of our conservative criteria, probably many more action potentials of this cluster are actually first action potentials in bursts). The bottom left cluster mostly represents the second to penultimate action potentials in bursts. This cluster suggest that there were many bursts with at least three action potentials per burst. The upper left cluster mostly represents the last action potentials in bursts. The final, upper right cluster mostly represents action potentials in tonic mode, and some in this cluster may include single action potentials in burst mode (19). The burst percentage for the population of pulvinar cells was 17.1 ± 4.1 ($n = 15$). The example that is shown for the pulvinar exhibited a significant degree of rhythmicity (Fig. 2*A Lower*). The oscillations occurred with a frequency of ≈3 Hz and represent a marked difference from the first-order neurons. Most (9 of 15) pulvinar neurons showed such rhythmicity, and six showed little evidence of this (data are not shown because an example is given below for a medial dorsal cell).

F2

Fig. 2 *B* and *C* shows two examples from the medial dorsal nucleus that were remarkably similar to those of the pulvinar. Both examples show considerable bursting, but the example of Fig. 2*B* shows some rhythmicity, whereas that of Fig. 2*C* does not. The burst percentage for the population of medial dorsal cells was 26.9 ± 4.1 ($n = 10$). This was not significantly different from the levels that were observed in pulvinar neurons. Of these cells, eight showed evidence of rhythmicity and two did not.

F3

Fig. 3 *A* and *B* summarizes the extent of burst firing for cells of the different thalamic nuclei. Whether expressed as the percentage of action potentials in bursts (Fig. 3*A*) or in bursts per s (Fig. 3*B*), significantly more bursting was found in cells of the higher-order relays than in the first-order relays. These differences were statistically significant for all pairwise comparisons ($P < 0.01$ for all except those involving the ventral portion of the medial geniculate nucleus, where $P < 0.02$; Wilcoxon test this and other statistical pairwise comparisons). Fig. 3*C* shows that

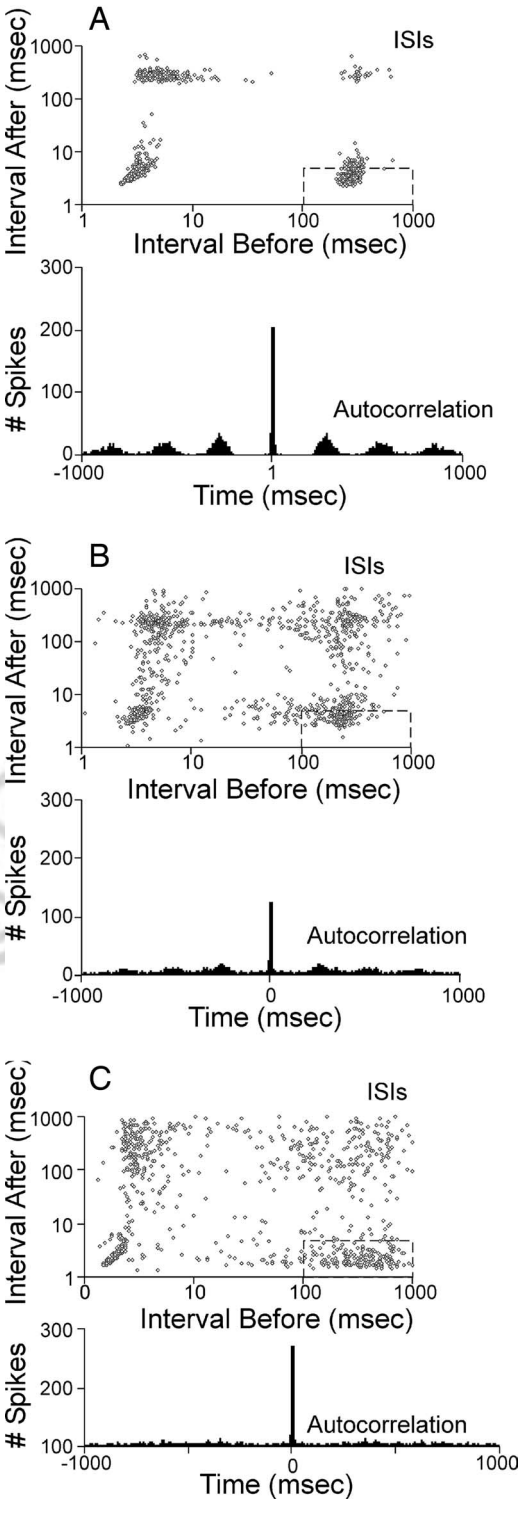

Fig. 2. Responses of neurons from higher-order thalamic relays showing two-dimensional interspike interval plots and autocorrelograms, as in Fig. 1. (*A*) Examples from a neuron of the pulvinar. (*B*) Examples from a neuron of the medial dorsal nucleus. (*C*) Examples from another neuron of the medial dorsal nucleus.

an opposite relationship exists for spontaneous activity, there being more for first-order relays than for higher-order ones, and the pairwise correlations again are statistically significant (all at $P < 0.01$). Fig. 3 also shows that there is no significant difference

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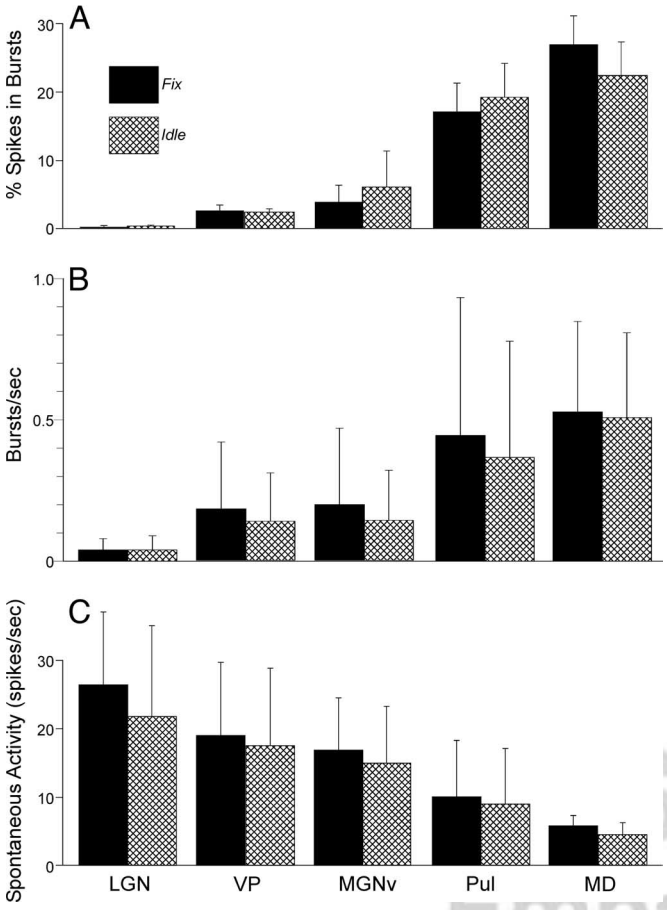

Fig. 3. Summary of bursting levels and spontaneous activity, showing mean and standard deviation for each group of thalamic neurons for both the fixation (*Fix*) and idle (*Idle*) conditions (see *Methods* for details). (*A*) Percentage of all action potentials found in bursts. (*B*) Spontaneous activity. (*C*) Burst rate shown as number of bursts per second. LGN, lateral geniculate nucleus; VP, ventral posterior nucleus; MGNv, ventral portion of the medial geniculate nucleus; Pul, pulvinar; MD, medial dorsal nucleus.

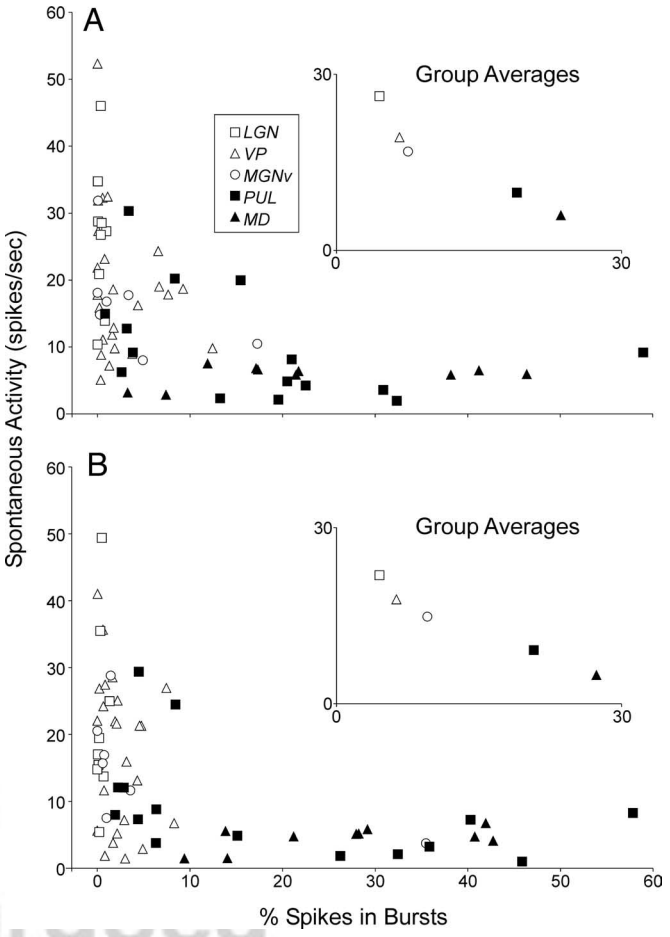

Fig. 4. Relationship between spontaneous activity and burst rate for individual neurons (large plots) and for group averages of each thalamic nucleus (small plots). Abbreviations are as defined in the Fig. 3 legend. (*A*) Fixation condition. (*B*) Idle condition.

either in amount of bursting or in spontaneous activity between the fixation and idle conditions ($P > 0.1$, for all comparisons). Note that cells of the lateral geniculate nucleus, which are the most intensively studied thalamic neurons, show the highest level of spontaneous activity and lowest level of bursting.

F4 Fig. 4 shows that, as suggested by Fig. 3 *A* and *C*, there are correlations for these cells between the burst levels (expressed here as percentage of action potentials in bursts) and spontaneous activity. This is shown separately for the fixation (Fig. 4*A*) and idle (Fig. 4*B*) conditions. Although they are within the samples from each thalamic nucleus, the correlations are not AQ: I significant; there is a significant relationship for all cells (fixation condition, $r = -0.49$ and $P < 0.001$; idle condition, $r = -0.48$ and $P < 0.001$). Fig. 4 *Insets* show the same relationship when the average values for each nucleus are plotted; here, too, the relationships are significant (fixation condition, $r = -0.95$ and $P < 0.01$; idle condition, $r = -0.98$ and $P < 0.001$).

Also, as observed from autocorrelograms, higher-order thalamic relay cells showed various levels of rhythmicity, whereas first-order nuclei showed little or no rhythmicity. Thus, even during alert states, rhythmic bursting has a profound role in higher-order thalamic relays.

Discussion

Extracellular recording of thalamic relay neurons of monkeys during an active visual-fixation task reveals that burst-mode activity is significantly higher for cells of the pulvinar and medial dorsal nucleus (two higher-order relays) than for cells of the lateral geniculate, ventral posterior, and ventral portion of the medial geniculate nuclei (three first-order relays). Overall, we found no difference in extent of bursting between the two higher-order relays. The lowest levels of bursting were detected in the lateral geniculate nucleus. We also noted differences in spontaneous activity among thalamic relays, such that those showing less bursting showed higher spontaneous levels. These same differences in bursting and spontaneous levels between relay nuclei were seen when the monkey sat idly in the chair and required no active fixation, and we saw no consistent or significant difference in response properties between behavioral conditions. Thus, one of the striking results is the difference in bursting levels between the two visual relays; cells of the first-order lateral geniculate nucleus showed much less bursting than cells of the higher-order pulvinar.

Patterns of Bursting. We saw two distinct forms of bursting: arrhythmic and rhythmic. There are several points to be made here. First, cells of the first-order relays showed only arrhythmic bursting, whereas those of the higher-order relays showed both forms of bursting. Note that these examples of rhythmic bursting were seen during fixation, which requires a high state of alertness, and thus, we can safely conclude that rhythmic bursting was fairly common in higher-order thalamic relays during alert wakefulness. This form of rhythmic bursting has been reported

for medial dorsal neurons in behaving monkeys, although it is not clear whether the bursts reported then are the same as those described here, being based on activation of T-type Ca^{2+} channels (30). We are not aware of any publication describing such bursting in monkey pulvinar. This ●● challenges the current dogma that rhythmic bursting dominates during slow-wave sleep and may be seen during drowsiness but not during wakefulness (31). Also, in our previous study of lateral geniculate cells (19), confirmed by the fact that only arrhythmic bursting was seen, we concluded that rhythmic bursting was not a feature of wakefulness. This conclusion, too, is refuted here, at least for some higher-order thalamic relays. Also, in ref. 19, we found that even during slow-wave sleep, rhythmic bursting was not as dominant as predicted from previous articles (31). Thus, what had been seen as a qualitative difference in thalamic cell responses between slow-wave sleep and wakefulness (slow-wave sleep dominated by rhythmic bursting and the wakefulness dominated by little or no arrhythmic bursting) is now challenged by the level of arrhythmic bursting that we have seen in the pulvinar and medial dorsal nucleus.

Differences Between First- and Higher-Order Thalamic Relays. Studies of thalamic relay cells consistently have found that application of cholinergic agonists depolarized them (32–34), mainly by the action of nicotinic and muscarinic (M)1 receptors. The main source of cholinergic input to thalamic relay cells comes from cells of the brainstem, mostly the midbrain tegmentum, and these cells generally become more active with increasing levels of behavioral arousal (30). The general conclusion from these observations is that increasing levels of wakefulness lead to more depolarization of relay cells, and depolarized relay cells are much more apt to fire in tonic mode. However, those earlier articles reporting cholinergic effects on relay cells were limited to first-order relays (namely, the ventral posterior and lateral geniculate nuclei) (32–34).

Cholinergic effects on relay cells were extended to a higher-order thalamic relay in a recent article by Mooney *et al.* (23). They showed that acetylcholine depolarizes cells of the lemniscal (ventral) dorsal medial geniculate nucleus (a first-order relay), confirming and amplifying previous findings, but they also showed that acetylcholine hyperpolarizes cells of the nonlemniscal medial geniculate nucleus (a higher-order relay), this effect being mediated by the action of M2 receptors. Preliminary data have extended these observations of Mooney *et al.* (23), suggesting that higher-order relays in general have many cells that are inhibited by cholinergic agonists by the action of M2 agonists (24). The prediction is that arousal and increasing wakefulness would lead to hyperpolarization of thalamic relay cells inhibited by cholinergic input; this ●● would remove inactivation of the T-type Ca^{2+} channels and, thereby, lead to burst-mode activity in this behavioral state. Thus, an apparently common difference between cells of first- and higher-order relays (namely, M2 receptors being rare among first-order relays but common among higher-order relays) provides an explanation for the results that we have found in behaving monkeys.

Another potential explanation derives from the study of Bokor *et al.* (25). This ●● showed that a GABAergic innervation from the zona incerta fairly selectively targets higher-order thalamic relays, bypassing first-order ones. Also, they showed that activation of this pathway commonly led to bursting in the higher-order-relay cells. Presumably, the mechanism is the same as described above; the extra inhibition removes inactivation of the T-type Ca^{2+} channels.

Extent of Bursting. There are two reasons to believe that we may have underestimated the extent of bursting, especially for the first-order relays. First, our estimates of bursting are based on spontaneous activity. Prior studies of the lateral geniculate nucleus (9) have shown that levels of spontaneous activity are much lower during burst mode than during tonic mode. That is, when in burst mode, a thalamic relay cell is often silent, and thus this period cannot be judged to be any particular firing mode, but such a silent neuron challenged with a visual stimulus will often respond with a burst. It may be the case that the same features apply to relay cells in other nuclei, and if so, our estimates from spontaneous activity severely underestimate burst firing.

Second, a recent study of lateral geniculate neurons of the anesthetized cat (35) indicates that typical artificial visual stimuli, such as visual noise, evoke significantly less bursting than more physiological natural scenes. This ●● suggests that bursting may be much more prevalent for geniculate neurons in behaving animals when naturally viewing the real world than earlier estimates based on using artificial, geometric visual restrained animals. Whether an effect analogous to activation by natural visual scenes is present in other, nonvisual thalamic relays remains to be determined.

Relationship Between Bursting and Spontaneous Activity. Guido *et al.* (9) showed in a study of cells of the lateral geniculate nucleus in anesthetized cats that there was a significant negative correlation between spontaneous activity and burst rate. We found the same relationship in our study (see Fig. 4), and thus, we can extend this observation to the monkey, the unanesthetized animal, and to relays cells other than those in the lateral geniculate nucleus. It is especially interesting that lateral geniculate relay cells show the lowest bursting and highest spontaneous activity. This ●● likely reflect the relatively high rate of firing of their retinal afferents, which could serve to depolarize them, leading both to higher firing rates as well as less spontaneous bursting, because the depolarization would inactivate the T channels. Perhaps the other thalamic nuclei that we studied, and particularly the higher-order ones, have lower levels of firing among their main driving inputs, although as noted above, inhibiting inputs from brainstem could serve to keep the higher-order relay cells more hyperpolarized, leading to more bursting and lower spontaneous activity.

Possible Significance of Increased Burst Firing for First- and Higher-Order Relays. One hypothesis suggested for cells of the lateral geniculate nucleus is that burst firing serves as a wake-up call for the detection of novel, previously unattended stimuli (4, 7). The basis for this ●● is fourfold (reviewed in refs. 36 and 37). (*i*) Stimulus detectability is higher during burst firing; (*ii*) burst firing provides greater activation of cortical neurons; (*iii*) there is a greater tendency for some lateral geniculate cells to burst to the first cycle of a repeating visual stimulus, with the ensuing cycles evoking mostly tonic firing; and (*iv*) bursting increases as the animal goes from a high state of vigilance to drowsiness to slow-wave sleep, suggesting that bursting is more common during general inattention. It seems to be straightforward to extend this view to the other first-order thalamic relays studied here: that is, bursting would signal the presence of a new, previously unattended sensory stimulus.

The extension of this view to higher-order relays requires first appreciating that they serve to relay information originating in one cortical area to another cortical area (36–38). This cortico-thalamo-cortical pathway starts with a subset of layer-5 cells in the first cortical area. Cells in these higher-order thalamic relays would be in burst mode, presumably because they have been hyperpolarized and have not recently been activated by their layer-5 input. Thus, a burst elicited by new layer-5 activity could serve to alert or "wake up" their cortical targets that a new wave of information is arriving. In this sense, the hypothesis of the role of burst mode remains essentially the same for the first- and higher-order relays studied here. Nonetheless, it remains just a hypothesis that requires further testing.

This view is also interesting in that our present results suggest the possibility that bursting is more prevalent in higher-order than in first-order relays. This may, as suggested above, might be the simple result of a subset of higher-order relay cells being hyperpolarized by cholinergic brainstem inputs. Why a subset of cells would be more commonly in burst mode in higher-order relays is a mystery, but it may be the case that wake-up calls alerting the target cortical areas are more important for cortico-thalamo-cortical pathways than for first-order relay of information from the periphery.

An important proviso to the extension of this idea to higher-order relays is the observation that much of the bursting seen in this study is rhythmic, and because rhythmic bursting was not observed in earlier studies of first-order relays that led to the notion of bursting as a wake-up call, it is reasonable to question it here. However, as long as higher-order relay cells do not burst synchronously, the generation of a synchronous set of bursts in related relay cells could have the same powerful effect in cortex as proposed for asynchronous bursting in first-order relays. Also, many higher-order relay cells burst asynchronously, and therefore, this proviso would not apply. The main concern is that rhythmic bursting might also mean that the relay cells also burst synchronously, and if so, it is not clear how relatively effective an additional burst caused by new information might be. The observation of rhythmic bursting in many higher-order relay cells suggests the possibility of further differences in processing between first- and higher-order thalamic relays.

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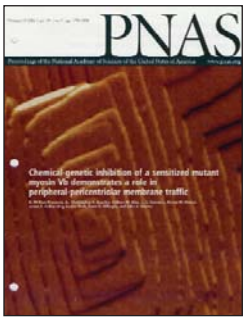

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