

# Cellular Mechanisms Underlying Activity Patterns in the Monkey Thalamus During Visual Behavior

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**Ramcharan, E. J., C. L. Cox, X. J. Zhan, S. M. Sherman, and J. W. Gnadt.** Cellular mechanisms underlying activity patterns in the monkey thalamus during visual behavior. *J Neurophysiol* 84: 1982–1987, 2000. We show for the first time with in vitro recording that burst firing in thalamic relay cells of the monkey is evoked by activation of voltage-dependent, low threshold  $\text{Ca}^{2+}$  spikes (LTSs), as has been described in other mammals. Due to variations in LTS amplitude, the number of action potentials evoked by an LTS could vary between 1 and 8. These data confirm the presence of two modes of firing in the monkey for thalamic relay cells, *tonic* and *burst*, the latter related to the activation of LTSs. With these details of the cellular processes underlying burst firing, we could account for many of the firing patterns we recorded from the lateral geniculate nucleus of the thalamus in behaving monkeys. In particular, we found clear evidence of burst firing during alert wakefulness, which had been thought to occur only during sleep or certain pathological states. This makes it likely that the burst firing seen in awake humans has the same cellular basis of LTSs, and this supports previous suggestions that burst firing represents an important relay mode for visual processing.

## INTRODUCTION

From studies of rodents and carnivores, we know that thalamic relay cells respond in one of two modes: *tonic* and *burst*. In tonic mode, a cell's firing rate fairly faithfully and linearly reflects the amplitude and duration of an excitatory input. In contrast, burst firing is characterized by high-frequency clusters of  $\leq 10$  action potentials and represents a less linear relay of its input. The switch in firing modes is dependent on the inactivation state of a voltage-dependent, transient, low threshold  $\text{Ca}^{2+}$  current known as  $I_T$  (Bal et al. 1995; Crunelli et al. 1989; Deschênes et al. 1984; Jahnsen and Llinàs 1984a,b). At depolarized membrane potentials,  $I_T$  is inactivated and the neuron responds in tonic mode. Hyperpolarization maintained for  $\geq 50$ – $100$  ms removes this inactivation, and a sufficiently large depolarizing current will then activate  $I_T$ . This, in turn, leads to a large, triangular, all-or-none  $\text{Ca}^{2+}$  spike that typically evokes a burst of action potentials riding its crest.

Although bursting tends to be rhythmic at 1–10 Hz during slow wave sleep or certain pathological states, like epilepsy (Steriade et al. 1993), recent studies in cats indicate that bursting appears arrhythmically during alert wakefulness and may provide an important form of information transfer to cortex that is less linear than tonic firing, but provides better signal detectability. In this regard, burst firing could provide a sort of “wake-up call” to

cortex signaling some potentially important change in the environment. Evidence for burst mode firing during wakefulness has now been reported for monkeys and humans (Lenz et al. 1998; Radhakrishnan et al. 1999; Ramcharan et al. 2000; Tsoukatos et al. 1997). Using in vitro recordings, we sought here to confirm at a cellular level that burst-like activity recorded extracellularly from thalamic relay cells in behaving monkeys also is related to a mechanism involving  $I_T$ .

## METHODS

**RECORDINGS IN THALAMIC SLICES.** Intracellular recordings were obtained in vitro from relay neurons of dorsal thalamus and the lateral geniculate nucleus of adult and juvenile macaque monkeys (2 rhesus and 3 cynomolgus) in compliance with approved animal protocols (e.g., the National Institutes of Health Guide for the Care and Use of Laboratory Animals). Briefly, animals were deeply anesthetized with pentobarbital sodium and a block of tissue containing the dorsal thalamus was removed, sliced (400–500  $\mu\text{m}$ ), stabilized, and prepared for intracellular recording according to standard protocols (Cox and Sherman 1999). Intracellular recordings were made in current clamp mode using fine tipped recording pipettes (40–100 M $\Omega$  filled with 1 M KAc and 2–3% neurobiotin). An Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) was used in bridge mode to record voltage signals. Throughout the recordings, an active bridge circuit was adjusted to balance the drop in potential produced by passing current through the recording electrode. Apparent input resistance was calculated from the slope of the linear portion of the current/voltage relationship. In some experiments, the  $\text{Na}^+$  channel blocker tetrodotoxin (TTX; 1  $\mu\text{M}$ ) was added to the bath to block action potentials. Following the recording, the slices were placed in 4% paraformaldehyde for overnight fixation, and standard protocols were used to reveal the neurobiotin and assess the morphology of the labeled cell with the light microscope.

**RECORDINGS IN THE BEHAVING MONKEY.** All in vivo experimental procedures were carried out according to standard protocols (e.g., Gnadt and Mays 1995). Chronic transcranial recording cylinders and scleral eye coils for recording eye position were surgically implanted under general anesthesia followed by several weeks recovery time. Eye movements were recorded relative to a fixed head position. As inducement to work, the daily intake of water was restricted to that earned as reward for successful completion of fixation trials. Each session, subjects were allowed to work to satiation. Tungsten microelectrodes (0.5–1.0 M $\Omega$ ) were lowered through the dura mater into the thalamus. Individual spikes, monitored on an oscilloscope and isolated through a time/amplitude window discriminator, were recorded

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by computer at a sampling time of 0.1 ms while eye position was monitored with a resolution of 2 ms temporally and  $0.2^\circ$  spatially.

Neurons in the lateral geniculate nucleus were identified by their relatively small visual receptive fields, their predictable ocular dominance switching as the electrode was lowered through the laminae, and a change to larger receptive fields with higher contrast sensitivity as we crossed from parvocellular (P) to magnocellular (M) laminae.

During fixation tasks, the receptive fields of the geniculate units were positioned on the screen of a visual display unit (Cambridge Systems VSG 2/3 graphics card) which 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.

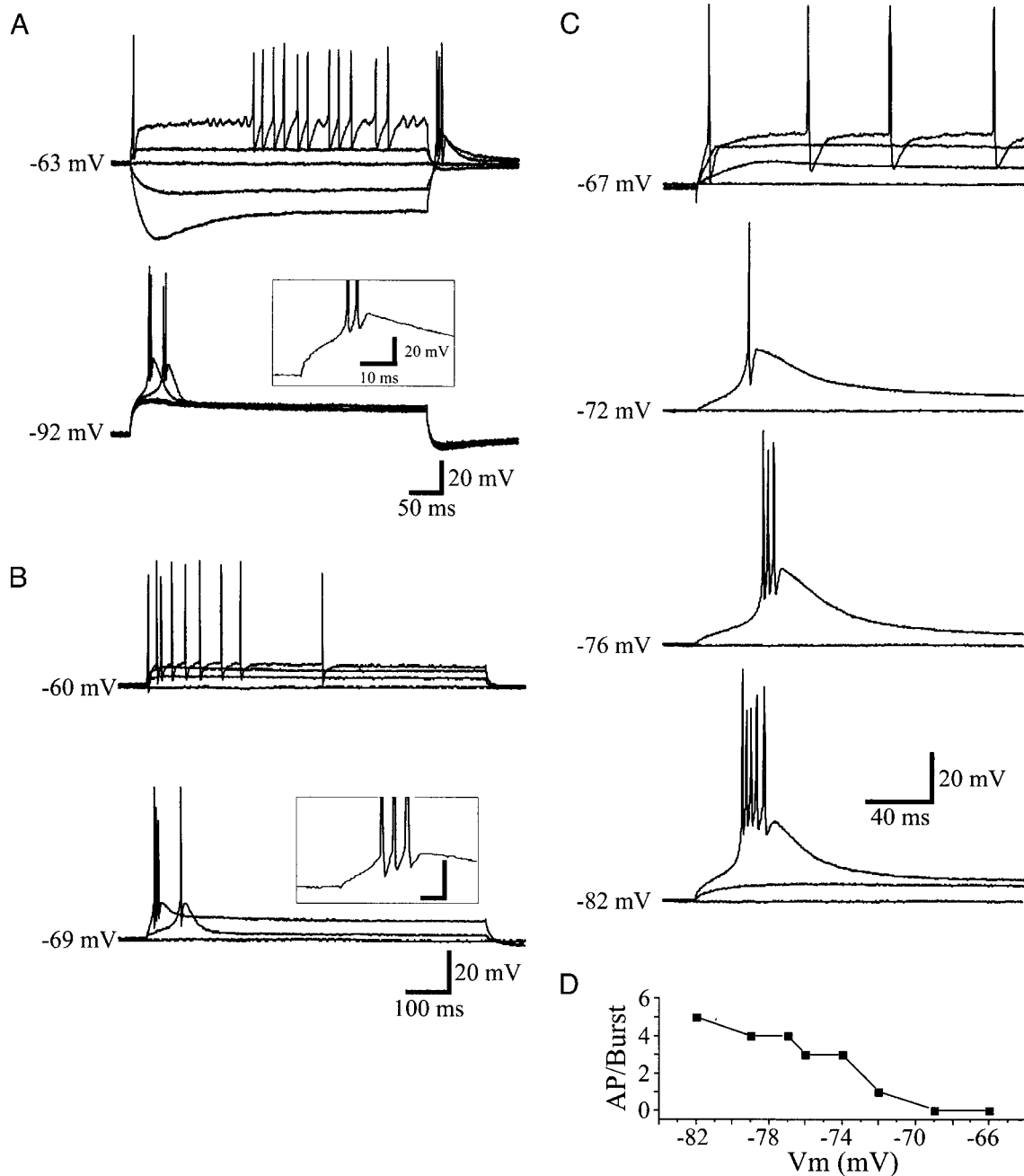


FIG. 1. Voltage dependence of burst firing. *A*: recordings from a lateral geniculate nucleus (LGN) relay neuron. The *top traces* show that at a relatively depolarized membrane potential ( $-63$  mV), where  $I_T$  is inactivated, the cell responds to depolarizing pulses with tonic discharge. Only with the depolarizing release from hyperpolarizing pulses which de-inactivates  $I_T$  are burst discharges observed. From a hyperpolarized Vm ( $-92$  mV), the depolarizing pulses evoke burst discharge. The *inset* depicts a faster time base illustrating the multiple action potentials during the burst. *B*: similarly, in a recording from a different dorsal thalamic neuron, a hyperpolarized Vm ( $-69$  mV) is required to evoke burst discharge. At the resting Vm ( $-60$  mV), only tonic discharge is evoked in response to depolarizing current pulses. *C*: the number of action potentials per burst discharge is voltage dependent. In a different cell, from a relatively depolarized Vm ( $-67$  mV) depolarizing current pulses evoke tonic firing. As Vm is hyperpolarized ( $-72$ ,  $-76$ , and  $-82$  mV), the stimuli evoke burst discharges with increasing numbers of action potentials per burst. *D*: plot of data from *C* illustrating the increase in action potentials per burst with hyperpolarized membrane potentials.

## RESULTS

*Properties of thalamic neurons in vitro*

We recorded intracellularly from 17 neurons in an *in vitro* slice preparation of the monkey thalamus; 7 were from the lateral geniculate nucleus and 10 from other, unspecified dorsal thalamic nuclei. All neurons exhibited a resting membrane potential more negative than  $-50$  mV ( $-62.1 \pm 6.1$  mV; mean  $\pm$  SD) and overshooting action potentials, and the input resistance of the population was  $26.5 \pm 14.2$  M $\Omega$ . Several of these neurons were injected with neurobiotin following recording, and three well stained cells were recovered histologically (see Fig. 2A).

Figure 1A shows that hyperpolarizing current pulses injected into a geniculate neuron produced a long latency depolarizing sag in the voltage trace. This is also seen in thalamic cells of rodents and cats, where the sag is due to activation of the hyperpolarization-activated mixed cation conductance,  $I_h$ . At the conclusion of the hyperpolarizing current step, the cell passively repolarized, and this led to a short, high-frequency burst of action potentials. Depolarizing current pulses from the

resting membrane potential of  $-63$  mV produced an ohmic response for smaller, subthreshold pulses, and with increasing stimulus intensity produced tonic firing (Fig. 1A). When the membrane potential of this cell was held at the more hyperpolarized level of  $-92$  mV, depolarizing current pulses evoked a burst of action potentials (Fig. 1A). Similar properties were observed in recordings from other thalamic nuclei as well (e.g., Fig. 1B). Thus we see that thalamic neurons in the monkey can discharge action potentials in both burst and tonic firing modes, and that the burst response requires initial hyperpolarization of the resting membrane potential.

In other mammalian species, burst firing is clearly voltage-dependent, requiring a relatively hyperpolarized membrane potential to de-inactivate  $I_T$  before a low threshold  $\text{Ca}^{2+}$  spike (LTS) can be activated. Figure 1C, taken from another geniculate neuron, shows this sort of voltage dependency for  $I_T$  and the LTS. From a depolarized membrane potential of  $-67$  mV at which  $I_T$  is inactivated, a depolarizing pulse evokes tonic firing. With initial hyperpolarization of the membrane potential to  $-72$ ,  $-76$ , and  $-82$  mV, subsequent depolarizing current

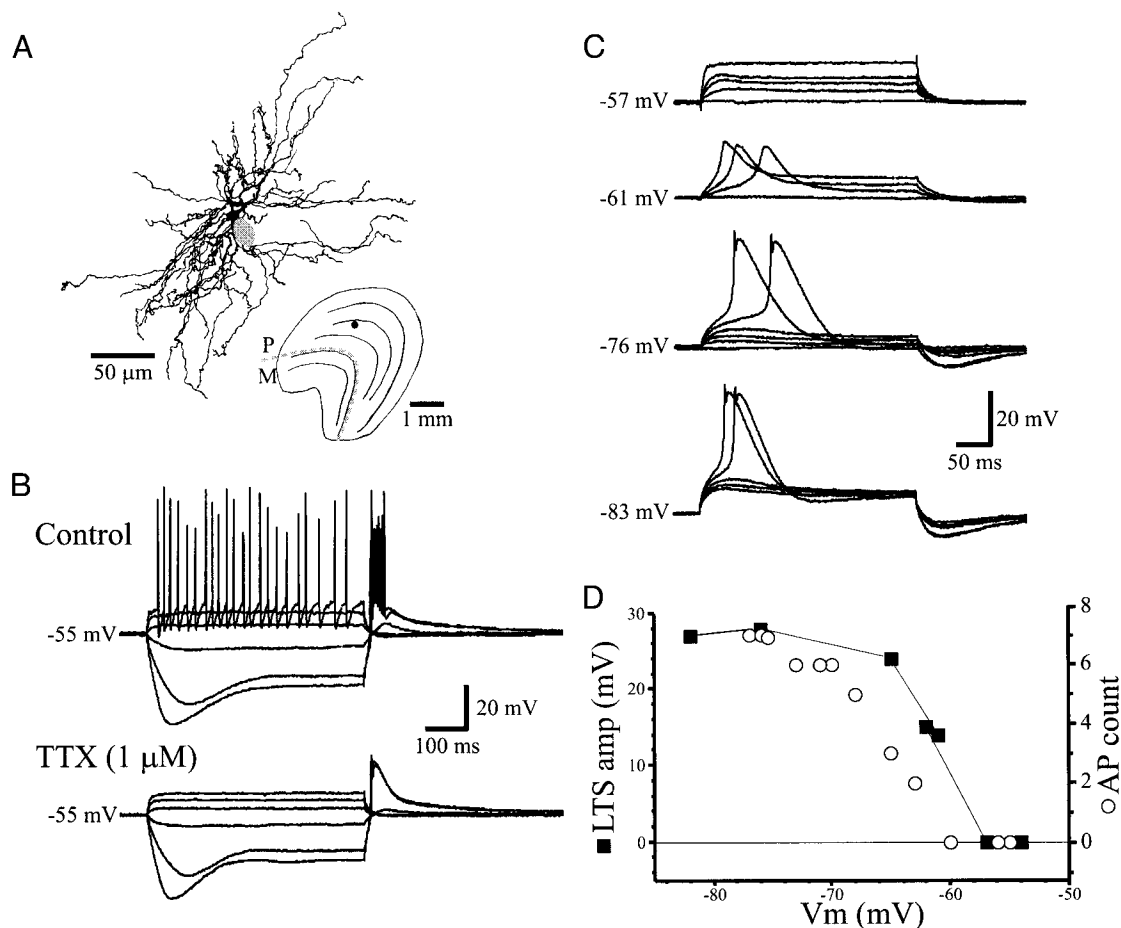


FIG. 2. Voltage dependence of low threshold  $\text{Ca}^{2+}$  spike (LTS) and burst output. *A*: camera-lucida reconstruction of an LGN relay neuron that was filled with neurobiotin. *B*: in control conditions, depolarizing pulses produced ohmic responses, and with increasing intensity evoked tonic discharge. Hyperpolarizing current pulses produced a depolarizing sag during the current injection, likely due to activation of  $I_h$ . Following offset of the hyperpolarizing current injection, a burst discharge is evoked. Note a similar action in tetrodotoxin (TTX;  $1 \mu\text{M}$ ) with the exception of an uncontaminated LTS that is evoked during offset of the current pulse.  $V_m = -55$  mV. *C*: in TTX ( $1 \mu\text{M}$ ), at the relatively depolarized  $V_m$  ( $-57$  mV), no LTS is evoked by depolarizing current pulses. However, small LTSs are produced from a  $V_m$  of  $-61$  mV, and larger LTSs are evoked as  $V_m$  is further hyperpolarized ( $-76$  and  $-83$  mV). *D*: relationship of data in *C*, showing the asymptotic increase in LTS amplitude and spikes per burst, as  $V_m$  becomes more hyperpolarized.

pulses evoked a characteristic LTS on which a high-frequency burst of action potentials rode. In addition, as has been documented for geniculate cells in the cat (Zhan et al. 2000), the number of action potentials per burst is related to the initial membrane potential: the more hyperpolarized this potential, the more  $I_T$  is de-inactivated, leading to a larger evoked LTS, which in turn evokes a larger burst of action potentials. Figure 1D summarizes this relationship. It is important to emphasize here that the relatively small LTS evoked from less hyperpolarized membrane potentials may activate a single action potential (e.g., the  $-72$  mV example of Fig. 1C).

Figure 2 shows data from another geniculate relay neuron that was labeled with neurobiotin after recording. The camera-lucida reconstruction (Fig. 2A) shows soma and dendritic morphology characteristic of thalamic relay cells described in other species. At resting levels, this cell responded to depolarizing current pulses with tonic firing but did produce burst firing in response to the termination of hyperpolarizing pulses (Fig. 2B, *Control*). TTX was then added to block action potentials and thus reveal the LTS more clearly (Fig. 2B, *TTX*). Figure 2C shows that the amplitude of the LTS depends on the initial membrane potential (cf. Fig. 1, C and D). At  $-57$  mV, only an ohmic response is seen to a depolarizing step, because  $I_T$  is largely inactivated at this membrane potential. However, from relatively hyperpolarized membrane potentials ( $-61$ ,  $-76$ , and  $-83$  mV), adequate depolarizing current steps evoke LTSs. There are two points to note about the LTSs evoked in Fig. 2C: 1) amplitude is greater for LTSs activated from more hyperpolarized initial membrane potentials; and 2) for any given initial membrane potential, the sizes of LTSs evoked are fairly constant. The latter point indicates that these LTSs are activated in a nearly "all-or-none" manner, as has been shown for thalamic relay cells in the cat (Zhan et al. 1999). The experiment shown in Fig. 2C was also performed on this cell before TTX was applied (data not shown), and Fig. 2D summarizes the dependence on initial membrane potential of both LTS amplitude (■) and number of action potentials in a burst (●). These data clearly indicate that the number of action potentials in a burst depends on the magnitude of the LTS.

#### Properties of thalamic neurons in the behaving monkey

We studied a total of 21 geniculate neurons in vivo (9 magnocellular and 12 parvocellular). For all in vivo recordings illustrated, the monkey actively fixated a small spot. During fixation, we quickly plotted the neuronal receptive field manually. All cells reported here were located  $10$ – $15^\circ$  from the fovea. We then applied a computer-generated, drifting sinusoidal grating onto the receptive field. Figure 3 shows data from three representative neurons in response to the grating. For each cell, we show a scatter plot of time intervals before each action potential versus the corresponding interval after each spike. To interpret the scatter plots, it is useful to keep in mind the temporal constraints for  $I_T$ : de-inactivation of  $I_T$  follows a complex function of time and membrane potential (Lu et al. 1992) which generally requires  $\geq 50$ – $100$  ms of hyperpolarization. Consequently, a silent period precedes each LTS. On the other hand, tonic firing has no such timing constraints since a tonic action

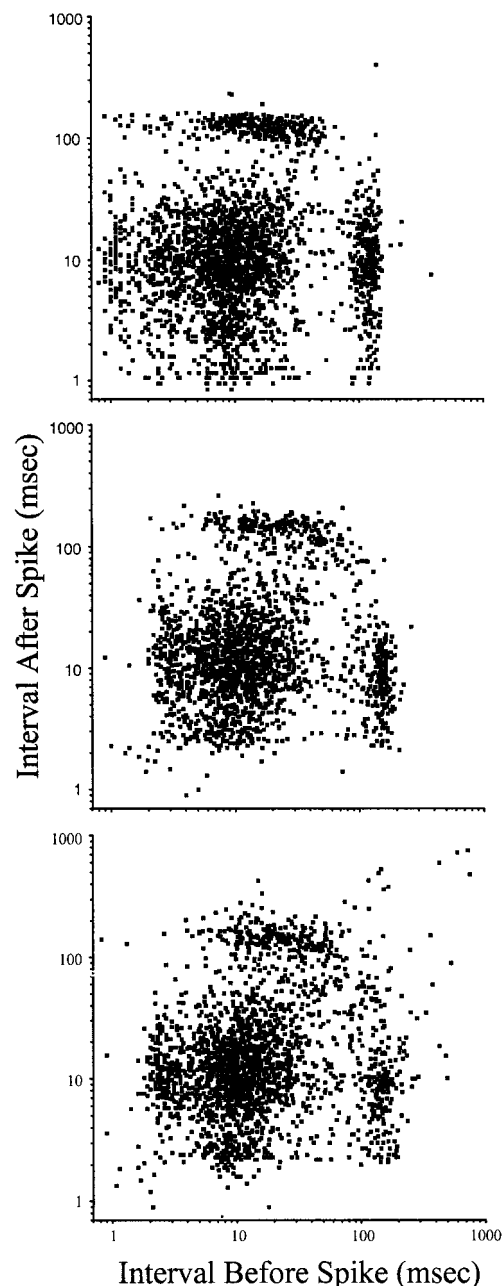


FIG. 3. Single cell recordings of three representative geniculate relay neurons while the animal fixated a small spot. The receptive fields of these cells were continually exposed to a sinusoidal grating drifted at 4 Hz. The scatter plots show the interval before a spike plotted against the interval after. The vertical band with prespike intervals  $\geq 100$  ms and postspike intervals in excess of 4 ms are likely to be single spikes in burst.

potential can follow or precede another by any interval greater than a refractory period of  $\sim 1$  ms.

As we have shown previously (Ramcharan et al. 2000), there are often three distinct clusters in the scatter plots for geniculate relay cells. The large, middle cluster of data points reflects tonic firing. A second cluster is a horizontal strip of points with postspike intervals  $\geq 100$  ms. Many of these points represent the last action potential before an LTS mediated burst. The third and particularly interesting cluster appears as a vertical band with prespike intervals  $\geq 100$  ms. Studies of cat geniculate cells have shown that virtually all action potentials with a



prespike interval  $\geq 100$  ms and a postspike interval  $\leq 4$  ms are the first action potential in a burst, with all subsequent action potentials having a postspike interval  $\leq 4$  ms being other members of the burst (Lu et al. 1992). However, these criteria are conservative and misidentify as tonic firing many action potentials that occur in bursts. We had suggested elsewhere (Ramcharan et al. 2000) that many of these spikes in the third cluster may represent LTSs that activate a single action potential. We have now confirmed in vitro that LTSs indeed may activate a single action potential following a prespike hyperpolarization, which could fall anywhere in the vertical cluster with a postspike interval  $> 4$  ms. We cannot be certain from the extracellular recording employed in vivo whether these action potentials represent single spikes riding a small LTS, tonic firing, or a mixture, but the point here is that they have a similar functional implication for signal detection and thalamocortical transmission.

#### DISCUSSION

The vertical band of action potentials following a preceding one by  $\geq 100$  ms has at least two functional correlates. First, the appearance of the preceding silent period indicates that the action potential appears on a background of very low spontaneous activity and will thus be more readily detectable than action potentials in the broad cluster of tonic firing that occur on a background of higher spontaneous activity (Guido et al. 1995; Sherman 1996). Second, thalamocortical synapses appear to have the property of *paired-pulse depression* (Agmon and Connors 1992; Stratford et al. 1996). That is, for an interval lasting for 10 s of ms, the second action potential in a pair in a thalamocortical axon evokes a reduced excitatory postsynaptic potential (EPSP) compared with that evoked by the first. This means that action potentials in the vertical cluster, appearing after a silent period long enough for paired-pulse depression to wear off, will evoke a maximum EPSP, whereas those in the broad central cluster will occur while the depression is still in force and will thus activate a smaller EPSP.

We also point out that a single action potential riding an LTS is functionally quite similar to a more traditional multiple spike burst. As noted above, both occur after a requisite silent period and would thus reflect comparable signal detectability (Guido et al. 1995; Sherman 1996). Furthermore, paired-pulse depression in the thalamocortical synapse implies that a single action potential on an LTS and the first spike in a burst will evoke a comparable EPSP, whereas succeeding action potentials in a burst will evoke depressed EPSPs. Thus the overall postsynaptic response may not be so different for these two conditions of burst mode firing. Finally, in terms of information theory, a burst behaves like a single event regardless of how many action potentials it contains (Reinagel et al. 1999). In other words, the first spike of a burst contains most of the information and other spikes add little additional information. Thus the information carried by a single action potential riding an LTS is very similar to that carried by a burst.

Regardless of how much of the vertical cluster of action potentials in Fig. 3 with prespike intervals  $\geq 100$  ms represents burst mode firing, it should by now be abundantly clear both that there is clear evidence of some burst mode firing during

awake, alert behavior in thalamic neurons of these monkeys and that this bursting is related to  $I_T$ . This conclusion is at odds with the historic view that thalamic relay cells fire strictly in tonic mode during wakefulness and burst mode during sleep (Livingstone and Hubel 1981; Steriade et al. 1993). There is now growing evidence that bursting can occur intermixed with tonic firing in the awake rat (Nicolelis et al. 1995), cat (Guido and Weyand 1995), monkey (Ramcharan et al. 2000), and human (Lenz et al. 1998; Radhakrishnan et al. 1999; Tsoukatos et al. 1997). Studies of the lightly anesthetized cat indicate that bursts can carry information roughly equivalent in magnitude to tonic firing (Reinagel et al. 1999), but that burst firing provides better signal detectability while tonic firing provides a more faithful relay of information. Given the close evolutionary relationship of monkeys to humans and the close similarities in the lateral geniculate nucleus between macaque monkeys and humans, these data strongly suggest that burst firing seen in awake humans (Lenz et al. 1998; Radhakrishnan et al. 1999; Tsoukatos et al. 1997) is also the result of activating  $I_T$ .

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