

## FUNCTIONAL IMPLICATIONS OF BURST FIRING AND SINGLE SPIKE ACTIVITY IN LATERAL GENICULATE RELAY NEURONS

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**Abstract**—Guinea-pig thalamocortical relay neurons can intrinsically generate action potentials in two distinct patterns: as high frequency bursts or as relatively independent single spikes. The burst firing mode is due to the presence of a low threshold  $Ca^{2+}$  current and imposes a marked non-linear transformation on depolarizing or hyperpolarizing inputs. In the burst firing mode, thalamic neurons respond to increasing frequencies of depolarizing inputs with progressively fewer action potentials such that they fail to respond to inputs arriving at rates greater than approximately 15 Hz. In this manner, the amplitude of the burst discharge relays little information concerning the characteristics of phasic excitatory postsynaptic potentials which may trigger them, but rather is determined by the membrane potential preceding the burst and the time interval since the last burst.

In contrast to the behavior of neurons in the burst firing mode, the pattern of action potentials generated after depolarization into the single spike mode is a more faithful representation of the characteristics of incoming excitatory postsynaptic potentials or depolarizing inputs. The pattern of action potentials generated in the single spike mode is determined by the intensity, duration, and frequency of incoming excitatory inputs even when they arrive at rates in excess of 100 Hz. These, and other properties, allow thalamic neurons to possess two distinct states of neuronal activity: an oscillatory mode in which rhythmic bursts of action potentials are generated and in which responsiveness to stimulation of peripheral receptive fields is greatly reduced, and a transfer mode in which action potentials are generated in relative independence of one another and in which the ability to respond to barrages of phasic excitatory inputs is greatly enhanced.

The presence of the rhythmic burst firing mode may therefore facilitate the filtering of sensory information during periods of drowsiness, inattentiveness, and slow wave sleep.

An important function of the thalamus is to regulate the transfer of sensory information from the periphery to the cortex. For example, in the mammalian visual system retinal ganglion cells innervate relay neurons in the dorsal lateral geniculate nucleus (LGNd) which in turn send their axons to the visual cortex.<sup>19</sup> Simultaneous recordings from retinal input and LGNd relay neuron output indicate that the fidelity with which synaptic inputs are transferred through the LGNd varies over a wide range, depending in part upon the arousal state of the animal.<sup>3,10,22,29,33</sup> The degradation of synaptic transfer through the thalamus during periods of decreased attentiveness and arousal has been attributed both to changes in the electrophysiological properties of thalamocortical relay neurons and to alterations in synaptic interactions, presumably in response to changes in extrathalamic influences.<sup>30,33</sup> The relative contribution of changes in electrophysiological properties versus synaptic interactions to these alterations in response properties of LGNd relay neurons has not been directly assessed.

Thalamocortical relay neurons display unique intrinsic electrophysiological properties which can dramatically alter their responsiveness to phasic synaptic inputs.<sup>17,18,32</sup> Thalamic relay neurons generate action potentials in one of two distinct modes: burst firing, in which 2–8 action potentials cluster together as a high frequency (200–400 Hz) burst discharge, and single spike activity in which action potentials occur singularly relatively independent of one another.<sup>6,18,32</sup> Burst firing, which is an intrinsic property of thalamocortical relay neurons, is largely restricted to periods of slow wave sleep or drowsiness, while single spike activity dominates during periods of waking, attentiveness, and rapid eye movement sleep.<sup>23,32,33</sup> Intracellular and extracellular microelectrode recordings from optic axons innervating the thalamus and from LGNd relay neurons indicate that the shift of thalamic activity from single spike to burst firing is associated with a dramatic decrease in the degree to which action potential output of the LGNd faithfully reflects incoming action potentials from the retina.<sup>3,33</sup> As a result, while synaptic inputs to the thalamus are accurately relayed to the cerebral cortex during periods of single spike activity, there is a substantial degradation of transfer when neurons of the LGNd are bursting.

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*Abbreviations:* EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; LGNd, dorsal lateral geniculate nucleus.

The electrophysiological basis for the two modes of action potential generation by thalamocortical relay neurons has been demonstrated by Jahnsen and Llinás<sup>17,18</sup> and Steriade, Deschênes and colleagues.<sup>8,32</sup> Thalamic relay neurons possess a substantial low-threshold  $\text{Ca}^{2+}$  current, the t-current, which underlies the generation of burst discharges.<sup>4,7,13</sup> At membrane potentials negative to approximately  $-65$  mV, phasic excitatory postsynaptic potentials (EPSPs) can activate the t-current, generating a low threshold  $\text{Ca}^{2+}$  "spike" which in turn triggers a high frequency burst of conventional  $\text{Na}^+$ - and  $\text{K}^+$ -dependent action potentials.<sup>10,11,17</sup> In contrast, at membrane potentials positive to  $-60$  mV, the t-current is inactivated and phasic synaptic inputs result in the generation of fast action potentials only if their amplitude surpasses action potential threshold (approximately  $-55$  mV).<sup>17,32</sup> Therefore the voltage-dependent properties of the t-current give rise to the anomalous situation in which membrane hyperpolarization can dramatically increase the action potential discharge of the neuron in response to a single phasic EPSP. Although this increase in action potential generation may be expected to increase temporarily the transfer of synaptic inputs through the thalamus, the relatively long refractory period of the t-current<sup>4,7,17</sup> may prevent thalamocortical relay neurons from responding accurately to a high frequency train of synaptic inputs, and therefore dramatically alter the pattern of action potentials received by the cerebral cortex in response to receptive field stimulation. This possible frequency limitation may have important functional consequences for the transmittal of sensory information from the periphery.

In this study we examine whether the intrinsic properties of LGNd neurons in the burst and single spike modes can affect the abilities of these neurons to respond to phasic inputs.

#### EXPERIMENTAL PROCEDURES

Techniques for preparing thalamic slices for intracellular recordings were similar to those published previously.<sup>25,26</sup> Male or female guinea-pigs were deeply anesthetized with sodium pentobarbital (30 mg/kg body weight) and decapitated. The brain was blocked anterior and posterior to the thalamus *in situ*, removed, and placed in physiological saline at  $5^\circ\text{C}$ . Four hundred-micrometer-thick coronal sections of thalamus were cut on a vibratome (Lancer Corporation). The lateral geniculate was dissected out of each slice and placed in an interface-style recording chamber and maintained at  $36^\circ\text{C}$ . The bathing solution was composed of (in mM): NaCl, 126; KCl, 2.5;  $\text{NaH}_2\text{PO}_4$ , 1.25;  $\text{MgSO}_4$ , 2;  $\text{CaCl}_2$ , 2;  $\text{NaHCO}_3$ , 26; glucose, 10. The bathing solution was saturated with 95%  $\text{O}_2$ -5%  $\text{CO}_2$  and its pH buffered to 7.4. All slices were allowed to recover inside the recording chamber for at least 2 h before recordings were obtained.

Intracellular recordings were obtained with glass microelectrodes formed from thin-wall capillary blanks (World Precision Instruments) on a Flaming-Brown P-80/PC puller, filled with 4 M KAc, and had a final resistance of 40–60  $\text{M}\Omega$ . All cells included in this study had overshooting action potentials and stable resting membrane potentials negative to  $-60$  mV. Activation of excitatory postsynaptic potentials was achieved through the delivery of electrical shocks

(100  $\mu\text{s}$  duration, 1–10 V amplitude) through a concentric stimulating electrode placed either in the white matter surrounding the lateral geniculate nucleus (which consists largely of optic tract axons) or within the nucleus near the recording electrode. Interpretation of response properties of LGNd neurons to activation of synaptic inputs with electrical shocks was complicated by the finding that these stimuli elicit not only phasic EPSPs, but also various phases of inhibitory postsynaptic potentials (IPSPs)<sup>6,15</sup> and slow EPSPs.<sup>9</sup> Therefore, the majority of data collected and illustrated here utilized the injection of depolarizing or hyperpolarizing current pulses in order to separate clearly response properties which were due to the intrinsic properties of thalamic relay neurons from those which were due to characteristics of synaptic potentials. The use of "square" pulses was chosen over sawtooth or sine wave functions since in the latter varying the frequency of the function also changes the rate of change of the injected current. Taking this difference into account, preliminary experiments with current injections which were either sawtooth or sine wave in waveform have yielded similar data to those reported here.

At rest, the membrane potential of thalamic neurons is typically positive to the voltage range in which burst responses can be elicited by a depolarizing input. However, repetitive burst responses can occur in response to a depolarizing current pulse after tonic hyperpolarization of the membrane potential (to approximately  $-75$  mV) or as rebound responses to hyperpolarizing current pulses (the distinction between the two paradigms being that in a hyperpolarizing current pulse the end, depolarizing phase of the pulse is necessarily the same amplitude as the initial hyperpolarizing phase). Rhythmic burst firing was tested using both of these paradigms. Data were encoded with a Neurocoder pulse code modulator encoding unit and recorded on videotape and on a chart recorder for later analysis.

#### RESULTS

Intracellular recordings were obtained from 29 guinea-pig LGNd neurons. A representative sample of 20 of these neurons had an average resting membrane potential of  $-66$  mV ( $\pm 3$ , S.D.), input resistance of 46  $\text{M}\Omega$  ( $\pm 12$ ) and action potential amplitude of 91 mV ( $\pm 6$ ). The electrophysiological properties of these cells were typical for thalamocortical relay neurons,<sup>27,32</sup> and therefore we refer to them as such.

#### *Firing modes of thalamic relay neurons*

As previously reported,<sup>17,18,32</sup> LGNd relay neurons respond to the intracellular injection of a depolarizing current pulse or activation of synaptic inputs in one of three basic manners depending on membrane potential (Fig. 1). At normal resting potentials, a relay neuron's response to the intracellular injection of a depolarizing current pulse is apparently passive, the properties of which reflect the capacity and resistivity of the membrane (Fig. 1, asterisk). Activating synaptic inputs at this membrane potential results in a typical EPSP which in most cases is followed by one or two phases of IPSP (not shown).<sup>6,15</sup> If the neuron is depolarized to  $-55$  mV, injecting the same intracellular current pulse, or activation of synaptic inputs, causes the neuron to generate a single action potential (Fig. 1,  $-55$  mV). In contrast, the response of the neuron after hyperpolarization to  $-76$  mV to

both the current pulse and the synaptic stimulation is marked by the generation of a low threshold  $\text{Ca}^{2+}$  spike which triggers a high frequency (300–400 Hz) burst of three fast action potentials (Fig. 1B). As mentioned above, the “burstiness” of relay neurons at hyperpolarized membrane potentials leads to the unusual situation that hyperpolarization can appear to increase, rather than decrease, the cell’s responsiveness to a depolarizing input (Fig. 1).<sup>17,32</sup> However, *in vivo* data indicate that the burst firing mode of action potential generation is associated with a large decrease in response of thalamocortical relay neurons to receptive field stimulation. We therefore addressed this apparent paradox by investigating the electrophysiological properties of cells in burst and single spike firing modes.

#### *Effects of varying amplitude and duration of injected current pulse*

Since the low threshold  $\text{Ca}^{2+}$  current which underlies the  $\text{Ca}^{2+}$  spike inactivates at membrane potentials positive to approximately  $-60$  mV, one would predict that prolonged depolarizations from a hyperpolarized membrane potential should give rise to only a single burst. Indeed, intracellular injection of a depolarizing current pulse while the cell was tonically hyperpolarized into the burst firing mode with the intracellular injection of current resulted in a single burst of action potentials at the beginning of the pulse regardless of the pulse’s duration (not shown). In addition, short duration pulses generate bursts which significantly outlast the duration of the intracellular current pulse and which are nearly identical to those generated by a prolonged current pulse.

In contrast to the above findings, intracellular injection of current pulses of increasing duration when the cell was tonically depolarized to near single spike firing threshold resulted in a progressively lengthening train of fast action potentials which showed no significant signs of spike frequency adaptation. This result illustrates that the number and timing of action potentials in the single spike firing mode accurately reflects the duration of the depolarizing input, while spike activity in the burst firing mode does not. An additional difference between the two modes of action potential generation is found in the amplitude of the current pulse required for their activation. Intracellular injection of short duration pulses which readily triggered single action potentials in the single spike firing mode were often too small in amplitude to result in the generation of a low threshold  $\text{Ca}^{2+}$  spike in the burst firing mode (Fig. 1A). This demonstrates that the generation of a burst of action potentials in the burst firing mode required the injection of longer and larger depolarizing current pulses. Similar results were obtained with the electrical activation of excitatory afferents. Small EPSPs (1–3 mV) could readily activate single action potentials when the cell was tonically depolarized to near  $-55$  mV, but often failed to reliably generate burst discharges after tonic hyperpolarization to any membrane potential in the burst firing range ( $-65$  to  $-105$  mV; not shown).

One similarity between the generation of single spikes and burst discharges is that they can both occur in an all-or-none manner.<sup>17</sup> For example, intracellular injection of a threshold current pulse with the neuron in the burst firing mode either results in the

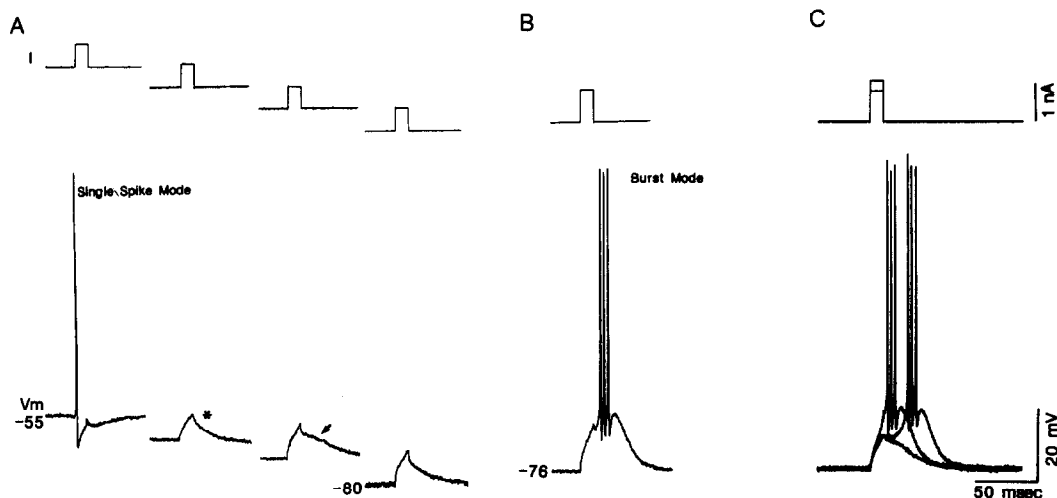


Fig. 1. The threshold properties for burst and single spike generation are different. (A) A neuron was progressively hyperpolarized with injected current from single spike mode to burst mode. At the most depolarized level ( $-55$  mV), a depolarizing current pulse caused a single action potential. At more hyperpolarized potentials, the same pulse caused only a passive depolarization or a small, presumed  $\text{Ca}^{2+}$  current which was subthreshold for burst generation (arrow). (B) Increasing the amplitude of the current pulse caused the same cell to fire a full burst from a hyperpolarized membrane potential. (C) A threshold current pulse caused a full burst in an all-or-none manner. Increasing the amplitude of the pulse shortens the latency of the burst, but does not affect its amplitude.

generation of a full burst of action potentials or only a largely passive response (Fig. 1C), as described previously.<sup>17</sup> Increasing the amplitude of the current pulse to suprathreshold reveals that the number and frequency of action potential generation does not change dramatically, but rather the burst occurs at a shorter latency (Fig. 1C). It is interesting to note, however, that the low threshold  $\text{Ca}^{2+}$  spike may vary over a large range of amplitudes and rates of rise and fall, depending largely upon the level of the membrane potential prior to its activation.<sup>17,18</sup> Although this is also true for the amplitude and rate of rise and fall of the fast  $\text{Na}^+$ - and  $\text{K}^+$ -mediated action potential, the sensitivity of these parameters to changes in membrane potential is not nearly as great.

#### *Frequency following capabilities*

Electrophysiological investigations of burst discharges in thalamic neurons<sup>8,17,18</sup> and of the underlying t-current<sup>4,7,13</sup> suggest that the amplitude of burst discharges should decrease as the time between bursts shortens due to the slow time course of removal of inactivation of  $I_t$ . To examine this possibility, we varied the rate at which relay neurons were stimulated in the burst mode by injecting depolarizing current pulses ( $n = 9$ ) or activating synaptic inputs ( $n = 4$ ). In both cases, thalamocortical relay neurons were not capable of generating recurring bursts of action potentials at rates greater than approximately 6–8 Hz (Fig. 2A, D). Furthermore, a significant decrease in the amplitude of the low threshold  $\text{Ca}^{2+}$  spike and a concomitant decrease in the number of fast action potentials generated by each was seen with depolarizing inputs or EPSPs which occurred at rates higher than just 1–2 Hz (Fig. 2). This property is well illustrated by varying the time interval between the injection of paired depolarizing current pulses (Fig. 2B). In this paradigm the amplitude of the low threshold  $\text{Ca}^{2+}$  spike progressively decreased as the interval between the first and second current pulse was shortened (Fig. 2B; see also Refs 8,17,18).

These results indicate that thalamocortical relay neurons in the burst firing mode are severely limited in their ability to follow recurring inputs at frequencies higher than approximately 8 Hz (Fig. 2D). In addition, the number of action potentials occurring in response to each input decreases dramatically as the frequency of inputs is increased (Fig. 2D). In contrast, in the single spike mode of action potential generation, thalamocortical relay neurons can faithfully follow, in a one-input-to-one-output manner, depolarizing pulses or synaptic potentials which occur at rates in excess of 100 Hz (Fig. 2C, D).

One of the basic assumptions concerning the transfer of information by neurons in the central nervous system is that the frequency with which action potentials occur conveys important information concerning the amplitude of the activating input. Comparing the frequency versus injected current ( $f$ - $I$ ) plots for the first interspike interval in a train of action poten-

tials generated in the single spike mode in response to a depolarizing current pulse revealed a highly linear relationship (Fig. 2E). The slope of this  $f$ - $I$  relationship was, on average, 131 Hz/nA ( $\pm 46$ ;  $n = 4$ ). In contrast, the relationship between spike generation in burst responses and amplitude of the depolarizing inputs was highly non-linear with the frequency increasing dramatically from zero to over 300 Hz around threshold for generation of a low threshold  $\text{Ca}^{2+}$  spike (Fig. 2E; 0.6 nA). Further increases in the amplitude of current injection after threshold for generation of burst discharge had been reached did result, however, in an increase in discharge frequency during the burst. The slope of this  $f$ - $I$  relationship was similar to that found when the same neuron is in the single spike firing mode (Fig. 2E).

#### *Effects of varying duration of hyperpolarization on frequency following capabilities of burst generation*

The ability of thalamocortical relay neurons to generate action potentials in response to rhythmic depolarizing current pulses in the burst firing mode appeared to be limited to an interburst frequency of approximately 6–8 Hz (Fig. 2D). However, *in vivo*, thalamocortical relay neurons have been found to generate rhythmic bursts of activity with interburst frequencies in excess of 12 Hz.<sup>23,32</sup> One possible explanation of this apparent discrepancy is that the stimuli used to collect our data were not optimal for rhythmic burst generation. In an effort to find more optimal stimuli, we varied the duration of the hyperpolarizing and depolarizing phases of a 5 or 10 Hz repeating current pulse (Fig. 3). Using this paradigm, we found that the ability of thalamocortical relay neurons to generate rhythmic burst discharges depended to a great extent on the relationship between the periods of hyperpolarization and depolarization (which in our experimental protocol were necessarily complementary). When the period of hyperpolarization was very short (Fig. 3A, B), the neuron failed to show rebound  $\text{Ca}^{2+}$  spikes, presumably owing to a lack of sufficient removal of inactivation of the t-current by the membrane hyperpolarization. As the duration of the hyperpolarizing phase was increased (and the duration of the depolarizing phase decreased) the effectiveness of the stimuli in generating burst activity gradually increased such that one, two and sometimes three action potentials were generated by the rebound  $\text{Ca}^{2+}$  spike (Fig. 3A, D, E). However, as the hyperpolarizing phase became longer, the depolarizing phase became very short in duration and was no longer capable of triggering a full  $\text{Ca}^{2+}$  spike, and therefore, the generation of action potentials ceased (Fig. 3F). The most effective stimulus for generation of the 10 Hz rhythm appeared to be a hyperpolarization of between 50 and 70 ms in duration, corresponding to a depolarization of 50–30 ms in duration ( $n = 4$ ). This finding indicates that our protocol of activating burst discharges with short duration (5–20 ms) depolarizing current pulses while

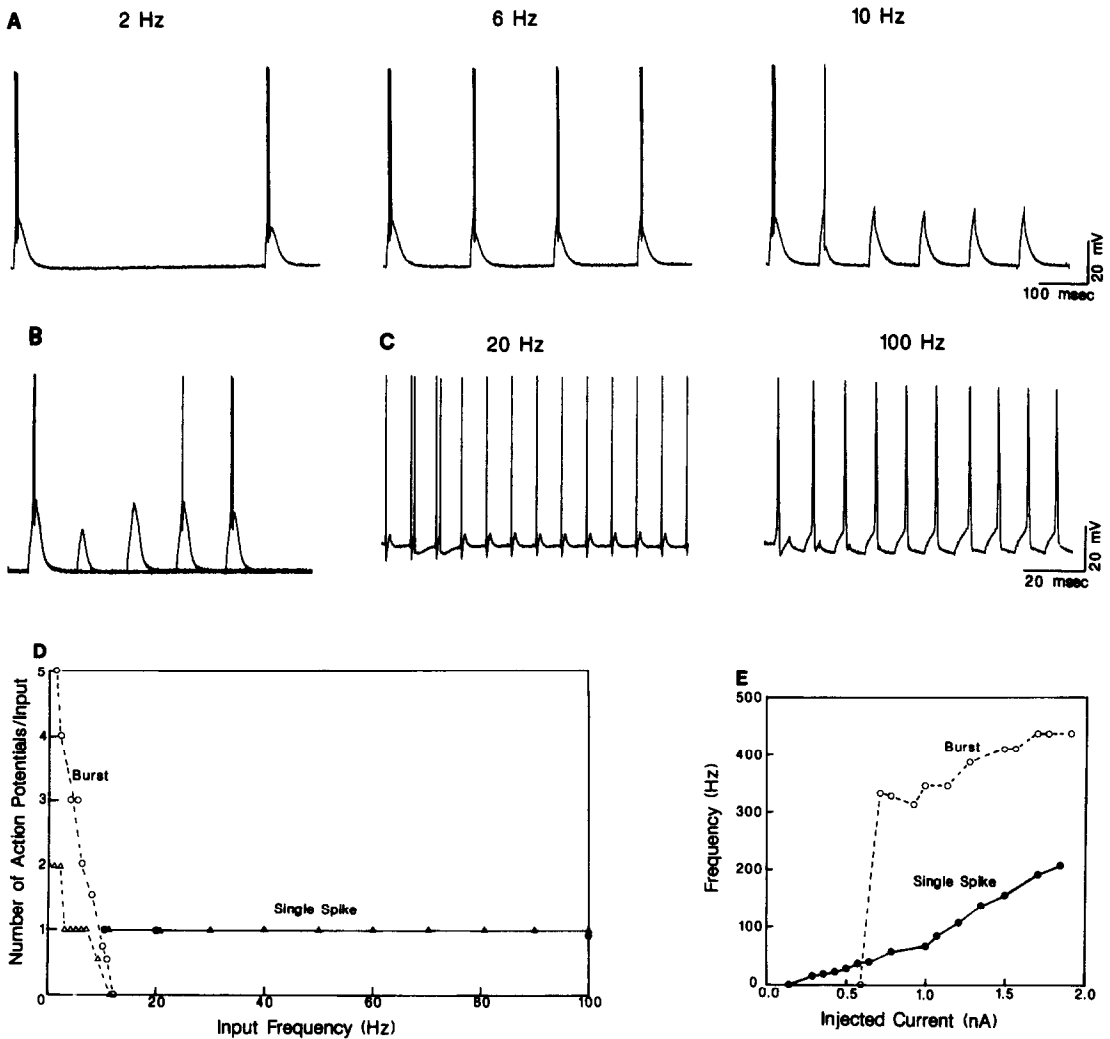


Fig. 2. The ability of thalamocortical relay neurons to follow varying frequencies of depolarizing inputs is different in the burst and single spike firing modes. (A) A relay neuron in the burst mode ( $-80$  mV) responded to depolarizing current pulses at 2 Hz by generating bursts of three spikes each. When the same neuron was stimulated at 6 Hz, the cell was able to follow the input, but the number of action potentials in each burst decreased from three initially to two at steady-state. At a 10 Hz stimulus rate, the neuron was only able to generate two bursts before its responses to each depolarization became largely passive. (B) When the interval between two depolarizing current pulses was increased, the neuron's response to the second stimulus changed from a passive depolarization to a full burst of four action potentials, thereby illustrating the refractory period of burst generation. (C) When maintained in the single spike mode ( $-63$  mV), the same neuron responded to depolarizing current pulses at 20 or 100 Hz by generating one, and occasionally two, action potentials per pulse. (D) Graphical representation of the data in (A) and (C) for two different neurons (circles and triangles) in either the burst firing (open symbols) or single spike (closed symbols) firing mode. (E) Frequency versus injected current ( $f-I$ ) plot for a neuron in either the burst firing or single spike mode of action potential generation. The frequency of action potential generation (as determined from the first interspike interval) increased linearly with the amplitude of a depolarizing input for cells in the single spike mode. The frequency of action potentials generated by the same neurons in the burst mode was highly non-linear, increasing from zero to over 300 Hz at the threshold for burst generation, which in this neuron was 0.6 nA.

the cell is tonically hyperpolarized into the burst firing mode is not the most effective manner in which to generate this type of activity. Indeed, using hyperpolarizing and depolarizing current pulses of various durations, we found that thalamocortical relay neurons could generate rhythmic burst discharges at interburst frequencies of up to approximately 15 Hz,

which is in agreement with the maximally observed interburst frequencies recorded *in vivo*.<sup>23,32</sup>

It is apparent from these, and other, data that the ability of thalamocortical relay neurons to generate rhythmic activity is a multifactorial process which is influenced not only by the duration of the hyperpolarizing and depolarizing phases of rhythmic

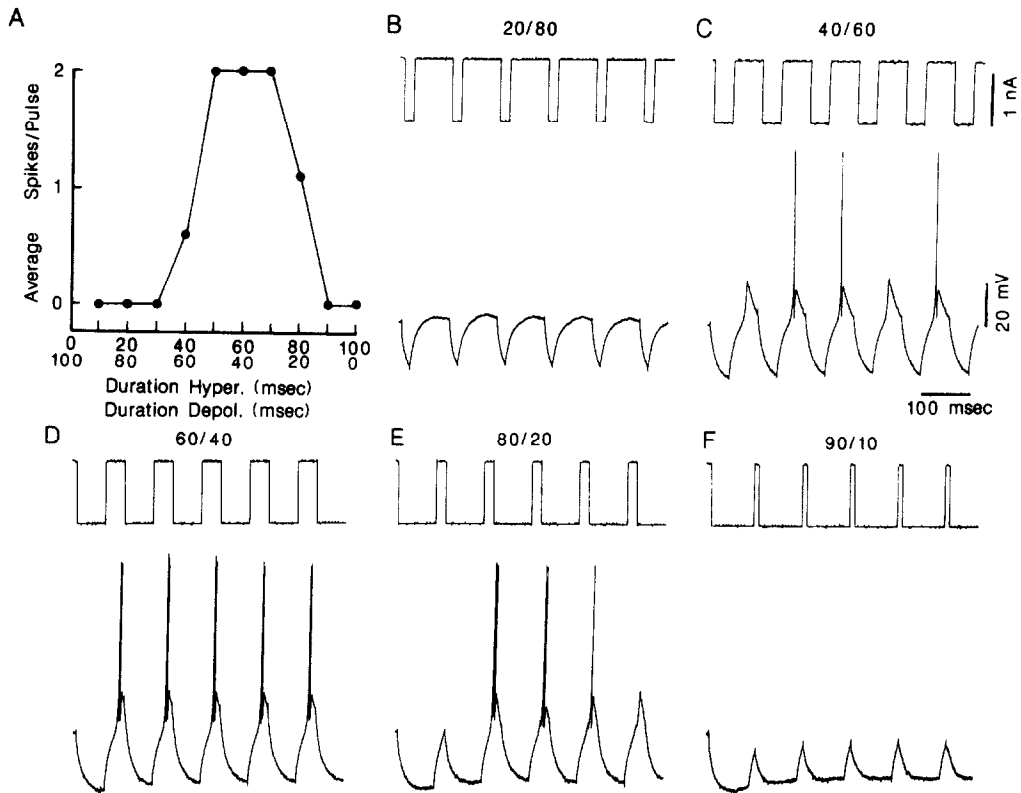


Fig. 3. The ability of relay neurons to rhythmically burst at 10 Hz depends upon the duration of the depolarization and the amount of time the cell was hyperpolarized prior to the depolarization. (A) A neuron at resting membrane potential ( $-65$  mV) received a  $1.2$  nA hyperpolarizing current pulse repetitively at 10 Hz. The duration of the hyperpolarizing and depolarizing phases was varied, and the number of spikes per pulse measured. The resulting graph shows that at a 10 Hz stimulation rate, relay neurons burst most effectively when they are hyperpolarized for slightly longer than they are depolarized. (B)–(F) The ability of this relay neuron to generate bursts during 10 Hz cycles of hyperpolarization and depolarization depended on the amount of time the neuron spent within each state during a cycle. The numbers above each trace indicate the duration of the hyperpolarizing and depolarizing phases, respectively.

inputs, but also by their amplitude, the membrane potential of the neuron (and hence the status of various ionic currents in the neuron), and the influence of various modulatory neurotransmitters.<sup>33</sup>

#### *Effects of long-duration inhibition on neuronal excitability*

Intracellular recordings in thalamocortical relay neurons *in vivo* indicate that at least some forms of rhythmic burst firing during electroencephalogram-synchronized sleep are associated with the arrival of large, rhythmically occurring IPSPs.<sup>16,32</sup> The occurrence of these IPSPs has been proposed to underlie, in part, the reduced responsiveness of thalamic neurons during the generation of rhythmic activity.<sup>1,30</sup> To examine the consequences of IPSPs on the generation of rhythms and the responsiveness of thalamic relay neurons to depolarizing inputs, we activated inhibitory afferents to these neurons with local electrical shocks (Fig. 4). Local electrical stimulation resulted in a typical sequence which consisted of an initial EPSP followed by two phases of IPSP, as reported previously.<sup>5,6,15</sup> The two phases of IPSP have been

reported to be mediated largely by an increase in  $\text{Cl}^-$  and  $\text{K}^+$  conductance, respectively, owing to release of GABA and the subsequent activation of  $\text{GABA}_A$  and  $\text{GABA}_B$  receptors.<sup>6,15</sup> These short duration postsynaptic potentials could also be followed by very long duration EPSPs, which are probably due to the release of modulatory neurotransmitters.<sup>9</sup> As the stimulus intensity was steadily increased, all components of the response steadily increased as well, including the late hyperpolarizing potential. Eventually, the late hyperpolarizing potential was of sufficient amplitude to be followed by a rebound, low threshold  $\text{Ca}^{2+}$  spike (Fig. 4A). At this point the late hyperpolarizing potential had an amplitude of 10–20 mV and a duration of 200–300 ms (Fig. 4). We believe that the late hyperpolarizing potential represents a large late IPSP and not a large  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductance for the following reasons: as the intensity of the stimuli was increased, the late IPSP steadily increased without any discontinuities, even at the point when action potentials began to be generated; although thalamic neurons possess a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current its duration is generally

too short to mediate the present hyperpolarizing potential<sup>17,18</sup> and dorsal lateral geniculate relay neurons appear to lack the slow  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current ( $I_{\text{AHP}}$ ) typical of other cell types (McCormick, unpublished observations); the generation of  $\text{Ca}^{2+}$  spikes alone did not result in any afterhyperpolarizing potential (e.g. see Fig. 4; rebound low threshold  $\text{Ca}^{2+}$  spike). However, since the identity of this potential was not rigorously tested here, we will refer to it merely as the late hyperpolarizing potential.

Electrical stimuli which resulted in a large late hyperpolarizing potential also resulted in a cyclic alteration in neuronal responsiveness to a short duration (5–20 ms) depolarizing current pulse (Fig. 4). The responsiveness of the neuron was lowest at the peak of the late hyperpolarizing potential (Fig. 4B), and steadily increased until it peaked at the time in which the low threshold  $\text{Ca}^{2+}$  spike would have occurred (Fig. 4D, E). Thus, as the time interval between the depolarizing current pulse and the activation of synaptic afferents was progressively in-

creased, the amplitude of the response to the depolarizing pulse became less until the peak of the hyperpolarization (Fig. 4B) after which the depolarizing potential began activating a burst of action potentials, presumably from activation of the low threshold  $\text{Ca}^{2+}$  current (Fig. 4C–E). The response of the neuron to the depolarizing current pulse was greatest if it occurred at about the same time that the rebound  $\text{Ca}^{2+}$  spike would have occurred (compare Fig. 4A and E). Further increases in latency resulted in the rebound  $\text{Ca}^{2+}$  spike occurring where it normally would and the response to the depolarizing current pulse appearing to be passive (Fig. 4F). These results indicate that during rhythmic oscillation of the membrane potential in LGNd relay neurons, such as those which occur during electroencephalogram-synchronized sleep,<sup>8,16</sup> the response to retinal inputs will be greatest if the incoming EPSPs are synchronous with and of the same frequency as intrathalamic rhythms. Otherwise, the rhythmic changes in excitability which are associated with relay neuron

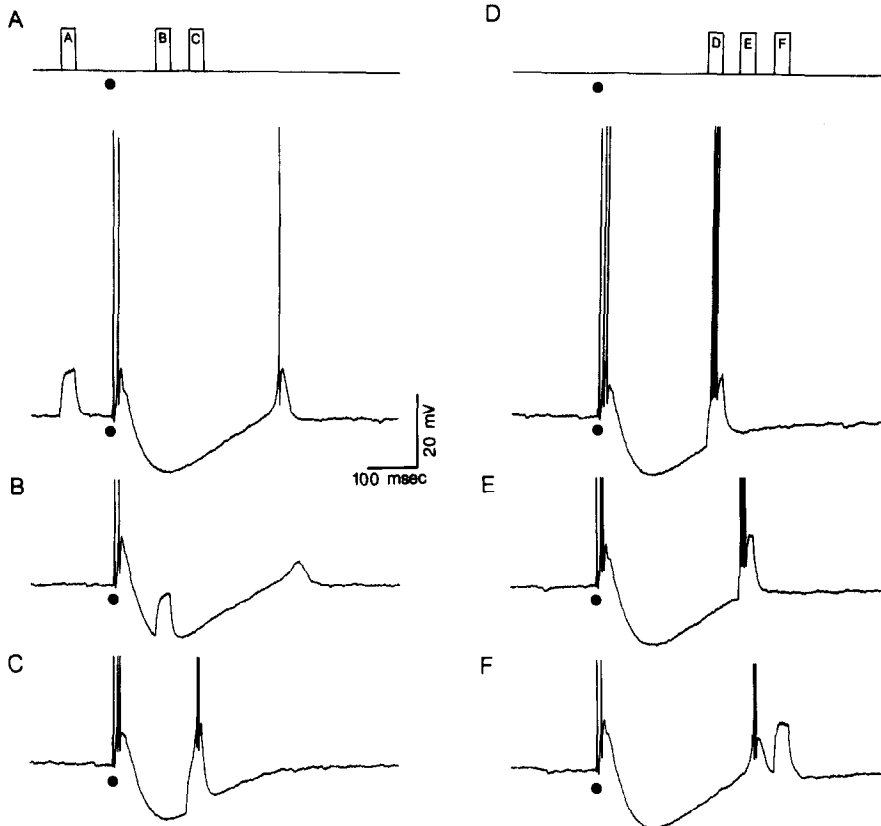


Fig. 4. Change in neuronal excitability during rhythmic oscillation of membrane potential. (A) Intracellular injection of a short duration (30 ms) depolarizing current pulse (A, upper trace labeled A) at resting membrane potential ( $-70$  mV) was followed by the delivery of a single electrical shock to the surrounding neuropil (dot). The activation of afferents resulted in a complex excitatory potential containing EPSPs, action potentials, and presumably low threshold  $\text{Ca}^{2+}$  current. This excitatory potential was followed by a slow hyperpolarization which was followed by a rebound low threshold  $\text{Ca}^{2+}$  spike, which itself triggered one action potential (A). (B) Moving the depolarizing current pulse to occur at the peak of the hyperpolarization resulted in a smaller response as well as a reduction in the rebound  $\text{Ca}^{2+}$  spike. (C)–(E) Further increases in latency resulted in the activation of two to four action potentials by the depolarizing current pulse. (F) Injection of the depolarizing current pulse after the occurrence of the rebound  $\text{Ca}^{2+}$  spike resulted in the typical subthreshold response.

membrane potential oscillation may impose upon a constant input from the retina a similar oscillation. However, it is also possible that the arrival of a train of EPSPs from the retina will disrupt the occurrence of rhythmic oscillations. For example, as seen in Fig. 4B, injection of a depolarizing current pulse at the peak of the hyperpolarization substantially reduced the amplitude of the rebound  $\text{Ca}^{2+}$  spike, presumably owing to a decrease in amplitude and duration of the hyperpolarization (which would lead to a decrease in de-inactivation of t-current). These results support the hypothesis that rhythmic oscillations and tonic discharges are two antagonistic and mutually exclusive states of thalamic neuronal firing and responsiveness.

#### DISCUSSION

The presence of two distinct modes of action potential generation in thalamic relay neurons has important implications for the pattern of activity generated by these cells. The temporal and voltage-dependent properties of burst firing facilitate the generation of intrathalamic rhythms, while the single spike firing mode appears more suited for the processing and transfer of trains of synaptic inputs activated by stimulation of receptive fields.

The ability of thalamic relay neurons to generate action potentials in two different manners is due largely to the presence of a substantial low threshold  $\text{Ca}^{2+}$  current (t-current) which underlies the generation of burst discharges.<sup>8,17,18</sup> The voltage-dependent properties of the t-current are such that it is tonically inactivated at membrane potentials positive to approximately  $-60$  mV. At resting, or at depolarized membrane potentials, t-current is largely inactivated and the cell's response to phasic inputs is characterized by the generation of trains of single action potentials.<sup>4,7,17,18</sup> For burst firing to occur, the inactivation of t-current must be removed by hyperpolarization of the membrane potential.<sup>8,17,18</sup> However, the removal of inactivation of t-current is a multifactorial process, depending not only upon the length of time which the membrane potential has been hyperpolarized, but also upon the amplitude of the hyperpolarization and the time since the last generation of a low threshold  $\text{Ca}^{2+}$  spike.<sup>4,7,13,17</sup> Intracellular current clamp recordings *in vitro* indicate that at rest, the membrane potential must be hyperpolarized negative to  $-75$  mV for 150–180 ms in order to remove  $>90\%$  of the inactivation of the t-current,<sup>17,18</sup> a finding which is in general agreement with estimates of the time constant for removal of inactivation of the t-current in thalamic neurons (80 ms at  $37^\circ\text{C}$ ) as revealed by whole-cell voltage clamp recordings.<sup>4</sup> This time course of removal of inactivation contributes to the inability of relay neurons to respond to trains of incoming EPSPs at a rate of more than approximately 15 Hz. At frequencies higher than this, the neuron is not hyperpolarized for a sufficiently

long period to allow removal of inactivation of enough t-current to reach action potential threshold during the next depolarizing phase (e.g. Fig. 2), and therefore the generation of low threshold  $\text{Ca}^{2+}$  spikes fails.

#### *Rhythmic burst firing*

During slow wave sleep, thalamocortical relay neurons generate high frequency bursts of action potentials owing to the occurrence of low threshold  $\text{Ca}^{2+}$  spikes.<sup>33</sup> The generation of these low threshold  $\text{Ca}^{2+}$  spikes appears to occur in at least three different patterns: as a critical component of spindle oscillations (spindle waves appear in the electroencephalogram as 7–14 Hz oscillations which steadily increase and then decrease in amplitude); as rhythmic bursts of action potentials with an interburst frequency of approximately 1–4 Hz,<sup>12,20,26,31</sup> and as non-rhythmic events which occur in apparent independence of one another.

Spindle waves occur spontaneously during drowsiness and slow wave sleep.<sup>33</sup> In addition, when animals are in these behavioral states, thalamic oscillations in the frequency range of spindles can be triggered by activation of corticothalamic or sub-thalamic afferents.<sup>32,33</sup> The generation of spindle oscillations appears to result from the interaction of thalamocortical relay neurons with the GABAergic neurons of the nucleus reticularis thalami, or, in the case of the LGNd, the perigeniculate nucleus, and depends critically upon the anatomical connections of these two cell types, as well as the intrinsic electrophysiological properties of the neurons involved and the characteristics of the postsynaptic responses which are induced by the transmitters which they release.<sup>32,33</sup> During the generation of spindle rhythms, thalamic relay neurons exhibit rhythmic hyperpolarizations in the frequency range of 7–14 Hz which are believed to represent IPSPs induced by discharges of nucleus reticularis neurons. Relay neurons subsequently generate rebound  $\text{Ca}^{2+}$  spikes to a subset of these IPSPs, thereby resulting in a frequency of burst generation during spindle waves which can be substantially less than 7–14 Hz. This result suggests that the generation of spindle waves is a group phenomenon being determined by the aggregate discharges of local collections of thalamocortical relay neurons and their corresponding nucleus reticularis neurons.<sup>33</sup>

Data obtained in the present study would suggest that during periods in which spindle oscillations are prominent the most effective stimuli in activating thalamic relay neurons would be in the same frequency range as the spindle waves (e.g. 7–14 Hz; Fig. 4). Barrages of EPSPs arriving at higher frequencies may disrupt the occurrence of these rhythms by interrupting the hyperpolarizing phases (which are necessary for occurrence of rebound burst discharges), while at the same time, the intrathalamic rhythms will tend to impose their rhythmicity upon the afferent activity. In this manner, the arrival of

significant afferent activity from the retina and the occurrence of burst discharges may be mutually exclusive states. In support of this hypothesis, Fourment *et al.* have reported that sudden decreases in spontaneous retinal activity (in the dark) below a critical level are associated with the appearance of rhythmic burst discharges in LGNd relay neurons.<sup>10</sup> Likewise, the reappearance of significant spontaneous retinal activity is associated with the disappearance of rhythmic burst activity in the LGNd, suggesting that the continual arrival of moderate or high levels of excitatory synaptic inputs from the periphery is not consistent with the occurrence of intrathalamic rhythms.

In contrast to spindle rhythms, the 1–4 Hz rhythmic burst firing appears to represent an endogenous oscillation of thalamic relay neurons, being brought out by de-afferentation *in vivo*,<sup>31</sup> or isolation *in vitro*,<sup>12,26</sup> and can occur during slow wave sleep.<sup>20</sup> Although the physiological basis of this intrinsic rhythm is not yet known in detail, it appears to result from the interaction of low threshold  $\text{Ca}^{2+}$  spikes with other slowly occurring hyperpolarizing and depolarizing currents.<sup>26</sup> Although not yet studied, it is possible that this slow rhythmic activity may appear during periods of deep sleep. The slow interburst frequency characteristic of this activity (1–4 Hz) may impose severe frequency limitations on the responsiveness of thalamic neurons in this state (e.g. Fig. 2D).

Non-rhythmic burst discharges may have a number of origins, including as an occasional rebound response to a large IPSP during the generation of a spindle wave<sup>32</sup> or as a response to activation of sensory or cortical inputs during periods when the membrane potential of the cell is relatively hyperpolarized.<sup>14</sup> In the latter case, the properties of the ionic mechanisms underlying burst discharges would be expected to reduce the responsiveness of the neuron to repetitive and high frequency arrival of EPSPs (e.g. Fig. 2D).

#### *Reduced neuronal excitability during synchronized burst firing*

The reduction in thalamic relay cell responsiveness to peripheral receptive field stimulation during periods of drowsiness, slow wave sleep, and burst discharges has been attributed to both removal of direct facilitatory influences on these cells from ascending neurotransmitter systems from the brainstem, such as acetylcholine,<sup>25</sup> and an increase in intrathalamic inhibitory mechanisms, particularly of large, slow IPSPs.<sup>30,33</sup> In addition, intracellular recordings *in vivo* indicate that the shift from waking to slow wave sleep is associated with a steady hyperpolarization of the membrane potential from the level of tonic single spike activity to a membrane potential in which low threshold  $\text{Ca}^{2+}$  spikes, and therefore burst discharges, can occur.<sup>14</sup> During the generation of at least some forms of rhythmic burst activity, such

as spindle waves, these burst discharges are triggered as the rebound from presumed inhibitory postsynaptic potentials.<sup>32</sup>

Based upon these, and other, data we would like to propose the following scenario for the decrease in thalamic responsiveness to stimulation of peripheral receptive fields during the transition from desynchronized to synchronized electroencephalograms, such as may occur during the transition from waking to drowsiness or sleep. A decrease in vigilance appears to be associated with an overall decrease in firing rate of serotonergic, noradrenergic, histaminergic and perhaps cholinergic neurons in the brainstem, hypothalamus, and basal forebrain.<sup>2,21,34,35</sup> This reduction in firing rate results in a decrease in the actions of these neurotransmitters in the thalamus, thereby resulting in a removal of the tonic depolarization of thalamic relay cells and a decrease in inhibition of intrathalamic and nucleus reticularis interneurons.<sup>24–28</sup> The resulting hyperpolarization of thalamic relay cells removes the inactivation of the low threshold  $\text{Ca}^{2+}$  current while the reduction in inhibition of GABAergic interneurons may allow the reappearance of large amplitude and slow duration IPSPs.<sup>16,33</sup> Consequently, the reduction in extrathalamic inputs may allow the thalamus to relax into a state of intrathalamic rhythmic burst generation.

Together, these various factors will reduce the ability of thalamocortical relay neurons to respond to trains of EPSPs. Tonic hyperpolarization of the cell increases the amplitude of EPSPs required to reach single spike firing threshold, and therefore only the largest EPSPs in the train will cause the generation of this form of action potential. However, further hyperpolarization of the cell into the burst firing mode may allow EPSPs to generate action potentials by activating a low threshold  $\text{Ca}^{2+}$  spike, although we have shown here that the frequency of action potential generation within the burst will not accurately reflect the amplitude or duration of the EPSP which generated it, but rather will be determined by the membrane events which precede the burst (e.g. the duration and amplitude of a preceding hyperpolarization).<sup>8</sup> In addition, the threshold for burst generation is higher than for the generation of single action potentials, and therefore small EPSPs which were capable of generating spikes in the single spike mode may not be able to in the burst firing mode. Even if a barrage of large EPSPs or IPSPs were successful in generating burst discharges, the rate with which bursts can be generated is limited to less than approximately 15 Hz, and therefore moderate amplitude postsynaptic potentials arriving at frequencies greater than this will not result in action potential generation in LGNd relay neurons and therefore will not be transmitted to the visual cortex. The arrival of a barrage of large amplitude EPSPs while the cell is in the burst firing mode may result in the generation of a burst at the beginning of the response, followed by a switch of the neuron to the tonic firing mode,

although the transfer ratio (the ratio of number of incoming EPSPs to outgoing action potentials) will be lower during the tonic phase due to the hyperpolarized state of the membrane potential. In this situation the cell will be emphasizing the initial transient component of the barrage of EPSPs at the expense of more tonic components.

A number of other factors are also important for understanding the relative unresponsiveness of thalamocortical relay neurons to retinal EPSPs during drowsiness and electroencephalogram-synchronized sleep. The presumed reduction in release of acetylcholine will result in a decrease in inhibition of GABAergic interneurons, both within the nucleus reticularis and within the LGNd.<sup>24,27</sup> This will increase the amplitude of IPSPs, especially late IPSPs,<sup>16</sup> which arrive in relay neurons, and subsequently the pattern of action potentials generated in relay neurons will be under a stronger influence from these two types of inhibitory neuron. This increase in inhibition may result in two phenomena: (1) the increase in burst discharges of nucleus reticularis neurons may impose upon thalamocortical relay cells rhythmic barrages of large amplitude IPSPs which could disrupt the response of these cells to inputs from the retina; and (2) the increased excitability of

intrageniculate interneurons, especially within synaptic glomeruli, may decrease the ability of retinal EPSPs to depolarize relay neurons due to their shunting by powerful GABAergic IPSPs.<sup>30</sup>

#### CONCLUSION

From these data it is clear that the efficacy with which synaptic potentials from the retina are transmitted to the cerebral cortex by thalamocortical relay neurons is determined by a large number of factors, among which the intrinsic properties of thalamic neurons contribute greatly. The thalamus fulfils its critical role of regulating the flow of sensory and motor information to the cerebral cortex by utilizing the intrinsic properties of thalamocortical neurons as well as by modulation of synaptic interactions by extrathalamic inputs.

*Acknowledgements*—We would like to thank Hans-Christian Pape for helpful comments on this manuscript. This work was supported by the National Institute for Communicative Disorders and Stroke, the Jacob Javits Center in Neuroscience, the Joseph Klingenstein Fund, and a fellowship from the National Science Foundation (H.R.F.).

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(Accepted 6 June 1990)