

Abolition of Spindle Oscillations by Serotonin and Norepinephrine in the Ferret Lateral Geniculate and Perigeniculate Nuclei In Vitro

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Summary

The transition from sleep to waking is associated with the abolition of spindle waves in thalamocortical neurons and the GABAergic cells of the thalamic reticular/perigeniculate nuclei. We tested the possibility that norepinephrine (NE) and serotonin (5-HT) may abolish spindle wave generation through an enhancement of the hyperpolarization-activated cation current I_h in thalamocortical neurons. Local application of agents known to enhance I_h , including 5-HT, NE, the adenylyl cyclase activator, forskolin, and the β -adrenergic agonist, isoproterenol, to lamina A1 of the dorsal lateral geniculate nucleus resulted in an abolition of local spindle wave generation in thalamocortical neurons. The abolition of spindle waves was reversed by the local application of the I_h channel blocker, cesium. These results suggest that NE and 5-HT may abolish the generation of spindle waves through the modulation of I_h in thalamocortical neurons.

Introduction

The transition from slow-wave sleep to arousal and attentiveness is associated with the abolition of synchronized slow rhythms, such as spindle waves and delta waves, in thalamocortical systems and the appearance of tonic single-spike activity (reviewed by Steriade et al., 1993). Spindle waves are a prototypical example of slow-wave sleep oscillations and are characterized by a 1 s–3 s period of 7 Hz–14 Hz rhythmic burst firing in large numbers of thalamic neurons. These synchronized oscillations are generated through an interaction between the GABA (γ -aminobutyric acid)-containing neurons of the thalamic reticular/perigeniculate nuclei and the recipient thalamocortical neurons (Steriade and Deschênes, 1984; Steriade et al., 1993; von Krosigk et al., 1993). The transition from slow-wave sleep to waking is associated with an abolition of this rhythmic burst firing, presumably owing to the depolarization of the membrane potential and the subsequent inactivation of the low-threshold Ca^{2+} current underlying these bursts (Hirsch et al., 1983; Steriade et al., 1986). The widespread noradrenergic and serotonergic projections, arising from the locus coeruleus and raphe nuclei, respectively, are thought to contribute to this switch from rhythmic burst to the tonic firing mode in the GABAergic thalamic reticular/perigeniculate (PGN) and thalamocortical neurons (Jouvet, 1972; Steriade and Deschênes, 1984; de Lima and Singer, 1987a, 1987b; Steriade and Llinás, 1988; Steriade and McCarley, 1990; McCormick, 1992a). Indeed, the discharge rate of locus coeruleus and raphe neurons increases in anticipation of behav-

ioral arousal or awakening from slow-wave sleep (Trulsson and Jacobs, 1979; Aston-Jones and Bloom, 1981; Jacobs, 1986; Foote and Morrison, 1987; Pollard and Schwartz, 1987).

Investigations into the postsynaptic actions of serotonin (5-HT) and norepinephrine (NE) in thalamic neurons have revealed that they have in common the ability to modulate the activities of these cells through either the reduction of a resting “leak” potassium current, I_{KL} , or enhancement of the hyperpolarization-activated cation current, I_h , depending in part on the cell type studied (for review, see McCormick, 1992a). In PGN and thalamic reticular GABAergic neurons, activation of α_1 -adrenoceptors and 5-HT_{2/1C} serotonergic receptors results in membrane depolarization through a reduction in I_{KL} (McCormick and Wang, 1991). Functionally, this membrane depolarization results in an abolition of rhythmic burst firing and the promotion of single-spike activity in single thalamic reticular/PGN neurons (McCormick and Wang, 1991; Bal and McCormick, 1993). In contrast, in thalamocortical neurons, application of NE results not only in a decrease in I_{KL} through the activation of α_1 -adrenoceptors, but also in an enhancement of the hyperpolarization-activated cation current I_h , presumably through the activation of adenylyl cyclase (McCormick and Pape, 1990b). Application of 5-HT to thalamocortical neurons does not result in a reduction in I_{KL} , but does enhance I_h (McCormick and Pape, 1990b). The functional consequences of a positive shift in the activation curve for I_h by NE and 5-HT are unclear. In single thalamocortical neurons, the enhancement of I_h results in a small (1 mV–3 mV) depolarization and an abolition of intrinsic rhythmic burst firing, apparently through the prevention of the large hyperpolarizations that are necessary to sustain this mode of action potential generation (McCormick and Pape, 1990b). We have hypothesized previously that the reduction of I_{KL} and I_h both may result in the abolition of spindle waves in the transition from sleep to waking (McCormick, 1992a). The reduction of I_{KL} was proposed to do so through a depolarization of the membrane potential resulting in inactivation of the low-threshold Ca^{2+} current upon which the rhythmic burst firing that characterizes spindle wave generation depends. Enhancement of I_h was also proposed to be capable of abolishing spindle waves. Here, it was suggested that the enhancement of I_h in thalamocortical cells would reduce the amplitude of inhibitory postsynaptic potentials (IPSPs) resulting from burst firing in thalamic reticular/PGN cells, and therefore prevent the generation of rebound low-threshold Ca^{2+} spikes in thalamocortical cells (McCormick and Pape, 1990b; McCormick, 1992a).

To test this hypothesis, we examined the effects of neurotransmitters that are known to modulate the activities of I_{KL} and I_h on spindle wave generation in spontaneously spindling ferret dorsal lateral geniculate nucleus (LGNd) slices maintained in vitro (Bal et al., 1995a, 1995b). Here, we demonstrate that application to thalamocortical neurons of agents that are known to either enhance I_h or reduce I_{KL} block spindle wave generation,

while in the GABAergic neurons of the perigeniculate nucleus (PGN) the activation of noradrenergic and serotonergic receptors that decrease I_{KL} are able to block spindle wave generation. Portions of these results have appeared in abstract form (Lee et al., 1994, 1995, Soc. Neurosci., abstracts).

Results

Intracellular and extracellular recordings were obtained from the GABAergic neurons of the PGN and the thalamocortical neurons in lamina A1 of the LGNd in spontaneously spindling ferret thalamic slices maintained in vitro (see Figure 1). Simultaneous extracellular multiple unit and intracellular recordings from thalamocortical cells in lamina A1 of the LGNd revealed the synchronization of action potential bursts associated with spindle wave generation ($n = 8$) (Figures 1A and 1B). As reported previously (Bal et al., 1995a, 1995b), during the generation of spindle waves LGNd thalamocortical neurons received barrages of IPSPs at a frequency of 6 Hz–10 Hz and these IPSPs resulted in the generation of rebound low-threshold Ca^{2+} spikes (Figure 1B). Depolarization of single thalamocortical neurons with the intracellular injection of current resulted in inactivation of the low-threshold Ca^{2+} current, abolition of rebound Ca^{2+} spikes, and appearance of tonic action potential generation (Figure 1A, +DC), which was interrupted by spindle IPSPs. These effects did not noticeably affect the generation of spindle waves in the multiple unit recordings, even though the intracellularly recorded neuron no longer actively participated in spindle waves with the generation of rebound action potentials (Figures 1A and 1C).

Intracellular recordings from PGN neurons during spindle wave generation revealed that these cells generated repetitive (2 Hz–9 Hz) high-frequency (up to 500 Hz) burst discharges mediated by low-threshold Ca^{2+} spikes that were activated by the arrival of barrages of excitatory postsynaptic potentials (EPSPs) (Figures 1D and 1E), as reported previously (Bal et al., 1995a, 1995b). Depolarization of single PGN neurons with the intracellular injection of current resulted in an abolition of repetitive burst discharges and a switch to the single-spike mode of action potential generation ($n = 9$) (Figures 1D and 1F). The depolarization of a single PGN neuron did not noticeably affect the generation of spindle waves in the extracellularly recorded thalamocortical cells (Figures 1D and 1F), but did markedly affect how the recorded PGN neuron responded to the arrival of barrages of postsynaptic potentials (PSPs) during spindle wave generation. At resting membrane potentials, the PGN cell generated repetitive bursts of action potentials in response to the arrival of PSP barrages during spindle wave generation (Figure 1E). In contrast, the same PSP barrages exhibited complex effects when the PGN cell was in the tonic firing mode (Figure 1F). Typically, these PSP barrages resulted in phasic increases in the tonic discharge of PGN neurons, although phasic decreases could also occur (Figure 1F). Presumably, this mixture of excitatory and inhibitory responses in the tonic firing mode results from the mixture of EPSPs and IPSPs that

comprise the PSP barrages arriving in PGN neurons during the generation of spindle waves (Bal et al., 1995b), as well as the interaction of these PSPs with the generation of action potentials and afterhyperpolarizations in the PGN neuron. The marked decrease in action potential discharges generated during spindle waves either by thalamocortical cells or PGN neurons upon depolarization appears to result from the inactivation of the low-threshold Ca^{2+} current and suggests that application of neurotransmitters that result in similar depolarizations of the membrane in a local population of neurons may abolish spindle wave generation.

Effects of 5-HT and NE on Spindle Wave Generation

Through the application of various agonists to either lamina A1 of the LGNd or to the PGN, we found that modulation of either one of these sites could abolish the generation of spindle wave generation. For example, local application of 5-HT (500 μ M in micropipette) ($n = 5$) (Figure 2A) or NE (500 μ M) ($n = 11$) (Figure 3A) to the PGN while recording intracellularly from a thalamocortical neuron in lamina A1 revealed an abolition of the large phasic IPSPs associated with spindle wave generation and the appearance of a tonic barrage of many small (0.1 mV–3 mV) IPSPs that together resulted in a prolonged hyperpolarization (1 mV–3 mV) of the membrane potential of the thalamocortical cell (cf. Figures 2B and 2C). These IPSPs were too small to result in the generation of rebound Ca^{2+} spikes. This switch in amplitude and frequency distribution of IPSPs arriving in thalamocortical neurons suggested that application of 5-HT or NE to the PGN resulted in a shift in firing mode of PGN cells. Indeed, intracellular recordings from PGN neurons during the local application of 5-HT ($n = 5$) (Figure 2D) or NE ($n = 9$) (Figure 3B) revealed a membrane depolarization of 10 mV–23 mV and a switch in firing mode from burst generation during the generation of spindle waves to tonic single-spike activity during which spindle waves were not apparent.

Application of 5-HT (Figure 2F) (0.5 mM–1.0 mM in micropipette) to lamina A1 while recording intracellularly in a thalamocortical neuron also resulted in abolition ($n = 3$ of 10 slices) or diminution ($n = 4$ of 10 slices) of the spindle oscillations locally and an increase in apparent input conductance of the recorded thalamocortical neuron (Figure 2G). In the remaining cases ($n = 3$ of 10 slices), the local application of 5-HT to lamina A1 resulted in no obvious change in the characteristics of the spindle waves. Application of NE ($n = 15$) to lamina A1 while recording intracellularly in a thalamocortical neuron resulted in a 3 mV–10 mV depolarization of the membrane potential and an abolition of spindle wave generation, as indicated by the abolition of the spindle wave-associated IPSPs (Figure 3C). Intracellular recording from PGN neurons during the application of NE to lamina A1 revealed an abolition of spindle wave-associated barrages of EPSPs (Figure 3D).

Effects of Modulation of I_h on Spindle Wave Generation

Previous work has demonstrated that the activation of α_1 -adrenoceptors in PGN or LGNd neurons can result in

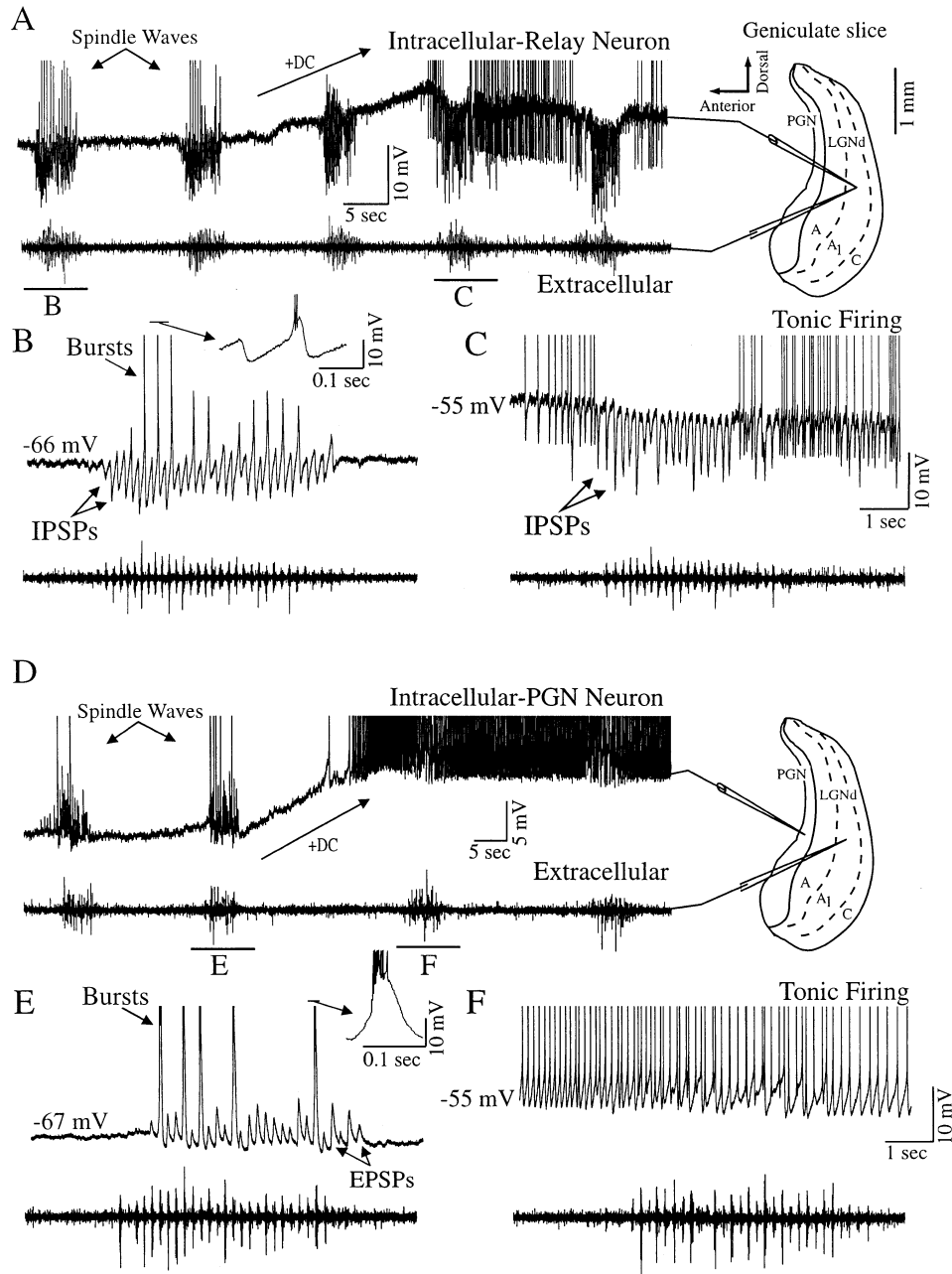


Figure 1. Voltage Dependence of Activities in LGNd Thalamocortical and GABAergic PGN Neurons during Spindle Wave Generation
(A) and (D) Simultaneous intracellular recording from either a LGNd thalamocortical neuron (A) or a GABAergic PGN neuron (D) and multiunit extracellular recording from thalamocortical neurons in lamina A1 of ferret LGNd demonstrated the occurrence of spontaneous spindle oscillations. The cartoon of the geniculate slice illustrates the locations of the electrodes. The response of the intracellularly recorded neurons during the generation of spindle waves while the neuron is depolarized with intracellular injection of current is illustrated.
(B) and (E) Illustration of one spindle sequence at an expanded time-base prior to intracellular injection of current. The LGNd thalamocortical neuron generated rebound low-threshold Ca^{2+} spikes following the arrival of IPSPs, while the PGN neuron generated low-threshold Ca^{2+} spikes and bursts of action potentials in response to barrages of EPSPs.
(C) and (F) Depolarization of the thalamocortical and PGN neuron with the intracellular injection of current results in an abolition of the low-threshold Ca^{2+} spikes and a marked reduction in the participation of the neuron in the spindle wave.

a depolarization from the reduction of a leak potassium conductance, I_{Kl} (McCormick and Prince, 1988; McCormick and Wang, 1991). In addition, the activation of β -adrenoceptors on thalamocortical cells can result in an enhancement of I_h (McCormick and Pape, 1990b). To

isolate the effect of β -adrenoceptor activation in thalamocortical neurons, responses to NE were investigated in the presence of α_1 - and α_2 -adrenoceptor antagonists prazosin (1 μM -5 μM in bath) and yohimbine (1 μM -5 μM in bath), respectively. In the presence of α -adrenoceptor

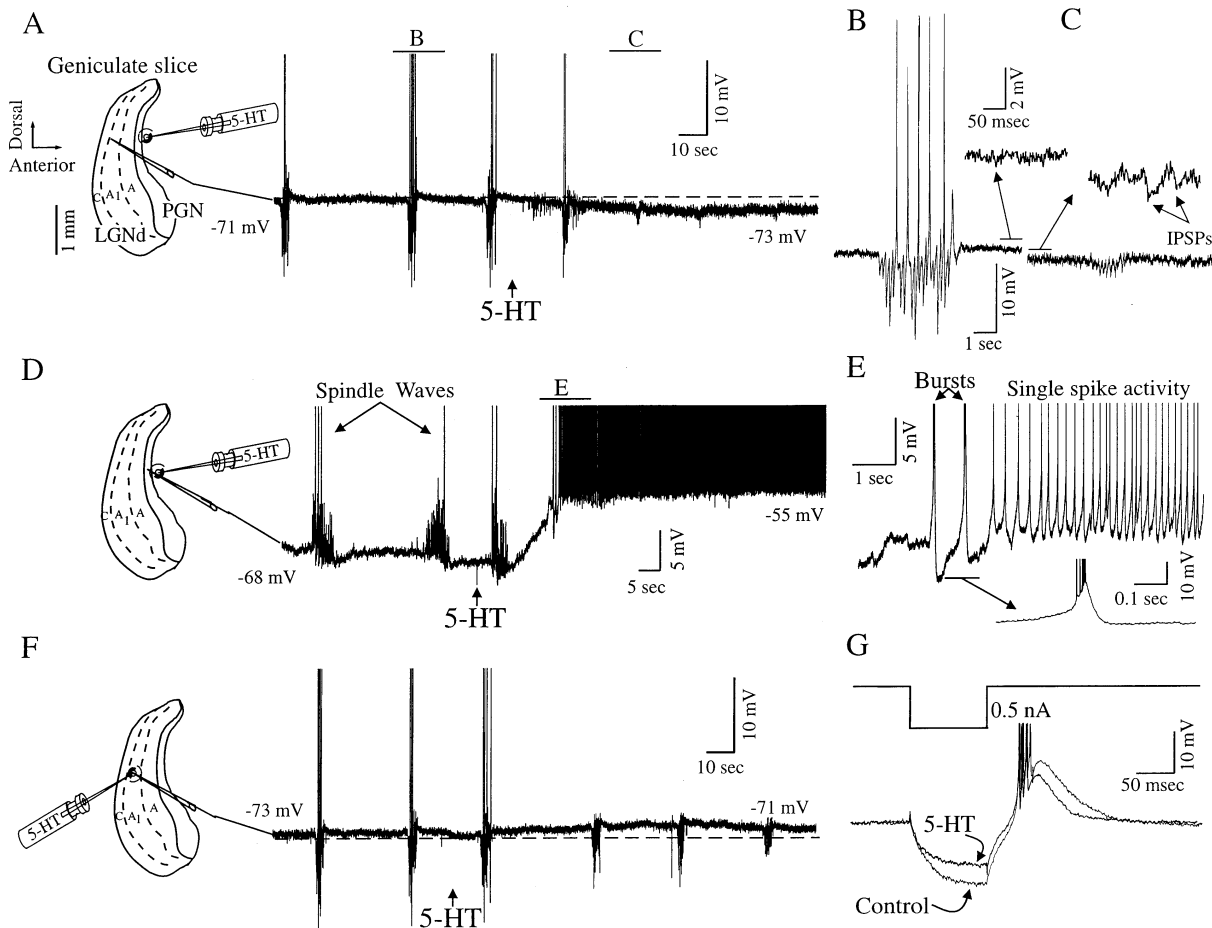


Figure 2. Application of 5-HT to Either the PGN or Lamina A1 Results in Cessation of Spindle Oscillations

(A) Local application of 5-HT to the PGN area immediately anterior to the intracellularly recorded thalamocortical neuron in lamina A1 results in the abolition of spindle wave generation and the generation of a slow membrane hyperpolarization of 2 mV characterized by the arrival of repetitive IPSPs.
 (B) Expansion of one spindle wave.
 (C) Expansion of IPSPs occurring after the local application of 5-HT.
 (D) Local application of 5-HT (500 μ M) near an intracellularly recorded PGN neuron resulted in membrane depolarization, occurrence of tonic firing, and block of spindle waves.
 (E) Expansion of recording as marked in (D) illustrates the occurrence of two bursts of action potentials followed by single-spike activity following the application of 5-HT.
 (F) Local application of 5-HT to the surface of the slice near the same intracellularly recorded LGNd thalamocortical neuron as in (A) resulted in slow membrane depolarization of 2 mV, abolition of rebound Ca^{2+} -mediated bursts, and diminished spindle IPSPs.
 (G) Examination of the electrotonic membrane response to 100 ms duration and 0.5 nA hyperpolarizing current pulses revealed that 5-HT application to this thalamocortical neuron resulted in an increase in apparent input conductance.

antagonists in bath, local application of NE to the PGN did not noticeably affect the generation of spindle waves recorded either in thalamocortical ($n = 3$) (Figure 3E) or GABAergic PGN cells ($n = 5$) (Figure 3F). In contrast, application of NE to lamina A1 resulted in an abolition of spindle wave generation, as recorded in both thalamocortical ($n = 7$) (Figure 3G) and GABAergic PGN cells ($n = 2$) (Figure 3H). These results suggest that NE abolishes spindle wave generation in the PGN through the activation of α_1 -adrenoceptors (see Figures 3A and 3B). In contrast, in the LGNd, NE retains the ability to abolish spindle wave generation, even in the presence of α_1 - and α_2 -adrenoceptor antagonists.

One possibility is that the activation of β -adrenoceptors on thalamocortical cells in the LGNd may also abolish the generation of spindle waves, while activation of

these receptors on PGN neurons may be without effect. To test this hypothesis, we examined the effects of the β -adrenoceptor agonist isoproterenol on thalamocortical and GABAergic PGN neurons. Local application of isoproterenol (50 μ M in micropipette) to the surface of the slice while recording from a thalamocortical neuron in lamina A1 resulted in a slow depolarization, an increase in apparent input conductance, and block of spindle waves ($n = 5$) (Figures 4A and 5B). In contrast, local application of isoproterenol to the PGN while recording intracellularly from a GABAergic PGN neuron did not result in any appreciable affect on spindle wave generation ($n = 4$) (Figure 4B). Application of isoproterenol to lamina A1 while recording from the same PGN neurons revealed a block of spindle wave generation ($n = 3$) (Figure 4C).

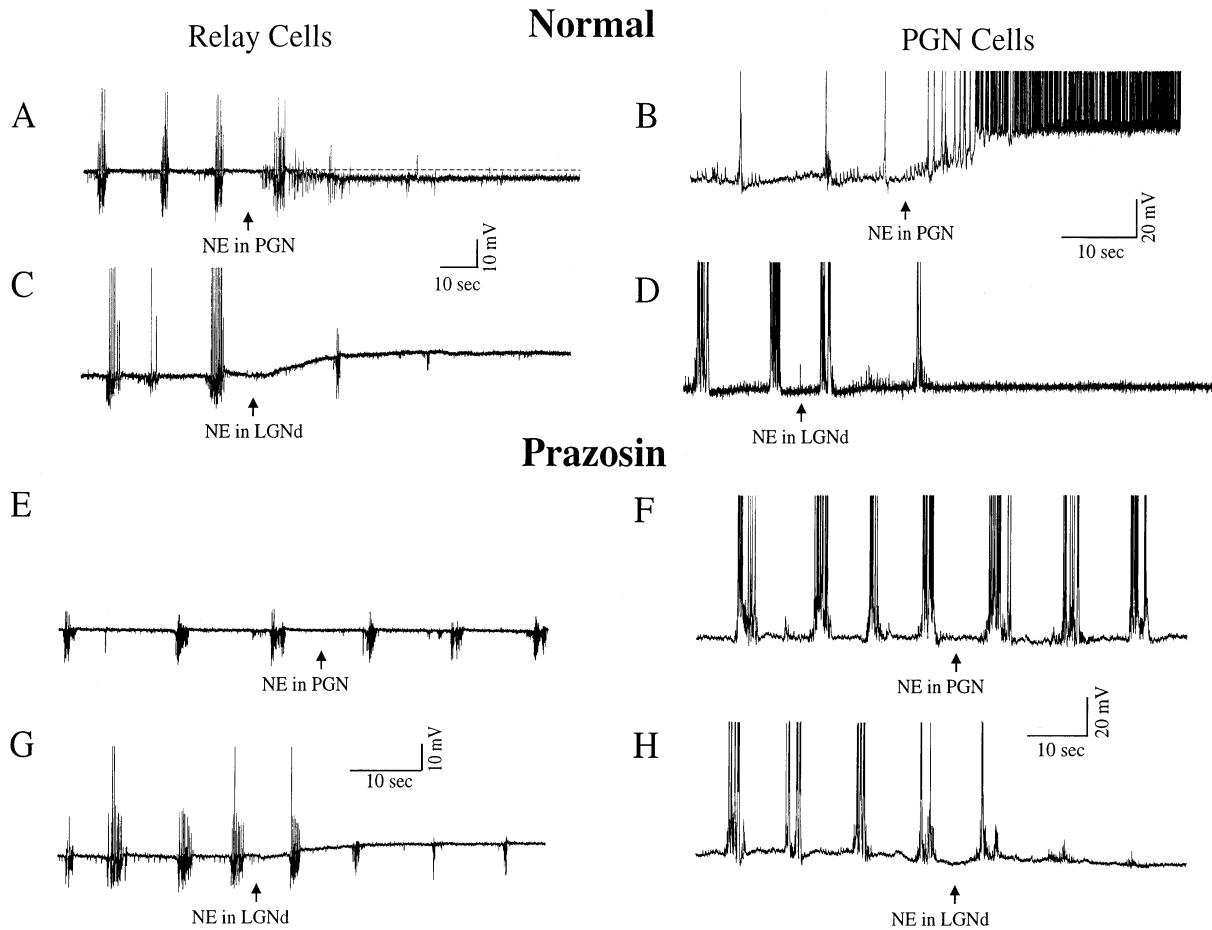


Figure 3. Effect of α_1 -Adrenoceptor Antagonist Prazosin (5 μ M) on the Responses of Thalamocortical Neurons and PGN Neurons to NE
 In normal solution, local application of NE (500 μ M) to the area of the slice limited to LGNd resulted in depolarization of the membrane potential and cessation of spindle wave generation in the thalamocortical neuron (C) while producing only a cessation of spindle oscillations without a change in membrane potential in the PGN neuron (D). Similarly, local application of NE to the area of slice limited to the PGN resulted in membrane hyperpolarization, appearance of 1 mV–2 mV IPSPs, and cessation of spindle oscillations in the thalamocortical neuron (A). In the PGN neuron, application of NE to the PGN resulted in membrane depolarization, appearance of tonic firing, and cessation of spindle oscillations (B). In the presence of prazosin, the application of NE to lamina A1 still antagonized spindle wave generation (G and H) while application to the PGN was without discernible effects (E and F).

To confirm that the action of isoproterenol was due to β -adrenoceptor stimulation, we examined whether the membrane depolarization, change in input conductance, and block of spindle waves induced by isoproterenol could be blocked by the β_1 -adrenoceptor antagonist, atenolol. In the absence of any antagonists in bath, local application of isoproterenol (50 μ M) to the PGN did not noticeably affect the generation of spindle waves, as recorded in a thalamocortical cell in lamina A1 (Figure 5A). However, local application of isoproterenol to lamina A1 within 50 μ m of the entry point of the intracellular electrode resulted in a slow increase in membrane conductance, a slow membrane depolarization of about 3 mV, and a cessation of spindle wave-associated IPSPs in thalamocortical neurons ($n = 6$) (Figure 5B). Closer examination of the electrotonic membrane response to the current pulse revealed that prior to isoproterenol application, the current pulse resulted in hyperpolarization of the membrane potential followed by a rebound Ca^{2+} mediated burst of action potentials (Figure 5B,

trace 1). After isoproterenol application, the current pulse resulted in a smaller amplitude hyperpolarization and no longer produced a rebound burst of action potentials, owing to the decreased amplitude of the underlying low-threshold Ca^{2+} spike (Figure 5B, trace 2). These responses to isoproterenol were attenuated markedly upon bath application of the β_1 -adrenoceptor antagonist, atenolol (50 μ M) ($n = 5$) (Figure 5C).

To test the hypothesis that these effects of isoproterenol may result from an enhancement of I_h , we examined whether the membrane depolarization and the block of spindle waves induced by β -adrenoceptor stimulation could be reversed by a reduction of I_h through local extracellular application of Cs^+ , a specific blocker of I_h (Mayer and Westbrook, 1983; McCormick and Pape, 1990a). As before, local application of isoproterenol (50 μ M in micropipette) to the surface of the slice near the intracellularly recorded and spontaneously spindling thalamocortical neurons in lamina A1 resulted in an ~ 3 mV membrane depolarization and cessation of spindle

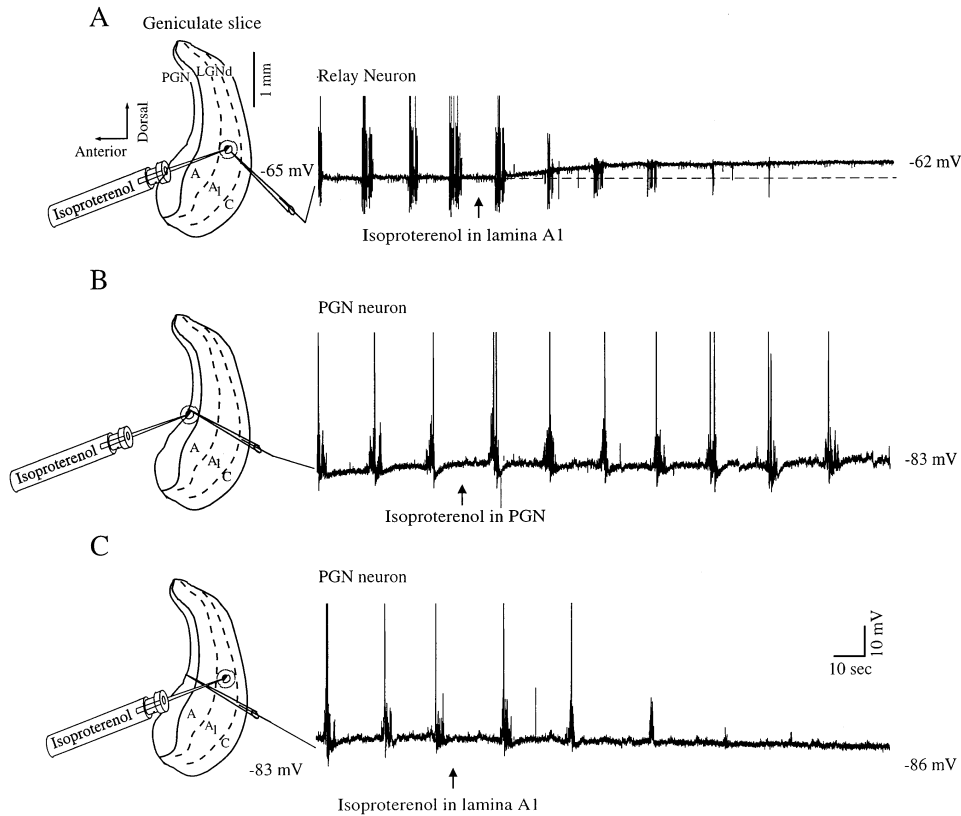


Figure 4. Application of the β -Adrenergic Agonist, Isoproterenol ($50 \mu\text{M}$), Resulted in Block of Spindle Oscillations Only when Applied to the LGNd and Not when Applied to the PGN

(A) Application of isoproterenol to lamina A1 while recording intracellularly from a thalamocortical cell results in the depolarization of the recorded neuron and the abolition of spindle waves.

(B) Local application of isoproterenol on the surface of the PGN while recording intracellularly from a PGN cell does not result in any appreciable change in spindle wave generation.

(C) Application of isoproterenol to lamina A1 results in an abolition in spindle wave generation, as recorded from the same PGN neuron as in (B).

oscillations ($n = 5$) (Figure 6A). The recovery from the application of isoproterenol was associated with a return to pre-isoproterenol membrane potential and the reappearance of spindle waves (Figure 6B, pre- Cs^+). Subsequently, local application of CsCl (10 mM in second micropipette) to the same area where isoproterenol was previously applied resulted in shortening of the interspindle interval until eventually an almost continuous spindle-like oscillation was seen ($n = 5$) (Figure 6B). As the CsCl washed out of the slice, the interspindle interval slowly lengthened until eventually it returned to pretreatment duration (Figures 6C and 6D). Upon complete washout of CsCl , isoproterenol was applied locally again to the surface of the slice, which, as before, resulted in an $\sim 3 \text{ mV}$ membrane depolarization and block of spindle oscillations (Figure 6C). During this blockade, the local application of CsCl resulted in a membrane hyperpolarization of $\sim 3 \text{ mV}$ back to the pre-isoproterenol membrane potential and the return of spontaneous spindle waves ($n = 5$) (Figures 6C and 6E). In another thalamocortical neuron, shortly after the local application of isoproterenol, which resulted in a 3 mV membrane depolarization and cessation of spindle oscillations, local application of CsCl resulted in return of spindle waves

(Figure 6F). The spindle waves ceased to occur when CsCl was discontinued to be locally applied, but then returned upon reapplication of CsCl (Figure 6F). This ability of Cs^+ to offset the effects of isoproterenol was dose-dependent. Prolonging the application of Cs^+ could not only reinstate the occurrence of spindle waves, but also block the spindle wave refractory period to the point where spindle waves may occur continuously (for example, see Figure 6B; see Bal and McCormick, 1996 [this issue of *Neuron*]). These results suggest that enhancement of I_h in thalamocortical neurons may reduce or block spindle waves and that these effects may be reversed by partial block of I_h channels through the extracellular application of CsCl .

To test further the possibility that enhancement of I_h may reduce or abolish spindle wave generation, we examined the effects of the adenylyl cyclase activator, forskolin, since this agent is known to enhance I_h in thalamocortical neurons (McCormick and Pape, 1990b). Simultaneous extracellular multiunit recording from the PGN and intracellular recording from a thalamocortical neuron in lamina A1 of the LGNd revealed the regular occurrence of spindle waves (Figure 7). Local application of forskolin ($500 \mu\text{M}$ in micropipette) to the surface

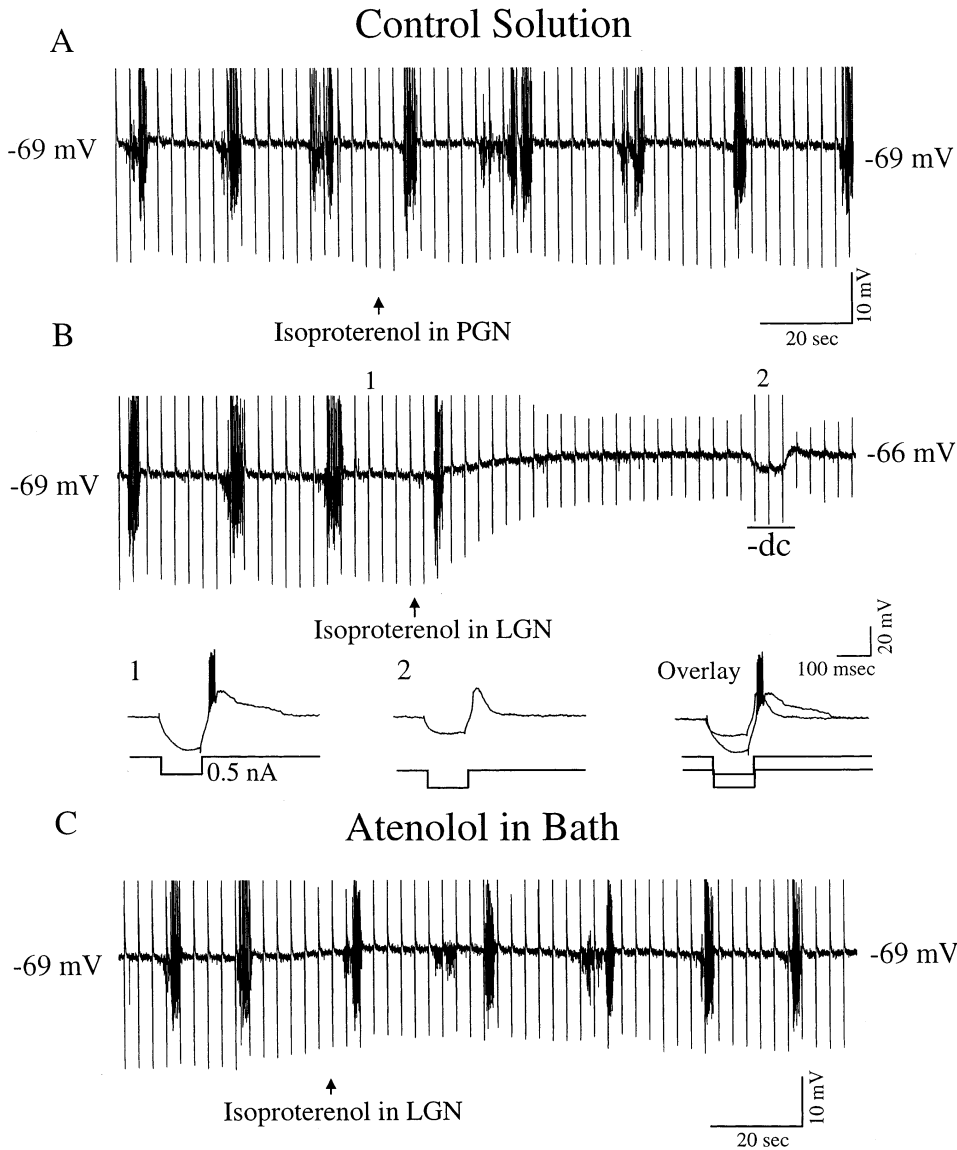


Figure 5. Atenolol Antagonizes the Action of Isoproterenol

(A) and (B) Application of isoproterenol to lamina A1 (B) but not to the PGN (A) results in abolition of spindle wave generation, as recorded in this thalamocortical neuron in lamina A1.
 (B) Traces 1 and 2, comparison of the electrotonic responses with constant hyperpolarizing current pulses reveals that the application of isoproterenol results in a large increase in apparent input conductance.
 (C) Bath application of atenolol (50 μ M) results in a substantial reduction in the postsynaptic response to isoproterenol and blocks its ability to abolish spindle waves.

of the slice near the entry point of the intracellular electrode in the LGNd resulted in a slow 1 mV–3 mV depolarization, a marked diminution of spindle wave-associated IPSPs, and an increase in membrane conductance in thalamocortical neurons ($n = 5$) (Figures 7A, 7C, and 7D). This diminution of spindle waves was also apparent in the extracellular recordings from the PGN (cf. Figures 7B and 7C). Local application of 1,9-dideoxy-forskolin (500 μ M in micropipette), a forskolin analog that only weakly stimulates adenylyl cyclase (Seamon and Daly, 1986), resulted in no effect on spindle oscillations in thalamocortical cells ($n = 5$). Subsequent applications of forskolin to these cells exhibited the full effect (data

not shown). In contrast with the effects of forskolin in the LGNd, local application of forskolin (500 μ M in micropipette) to the PGN did not have any discernible effects on spindle wave generation (Figure 7E).

Discussion

During slow-wave sleep, thalamocortical neurons and neuronal circuits generate rhythmic burst firing either as rhythmic low-threshold Ca^{2+} spikes in single thalamocortical neurons or as synchronized oscillations involving large numbers of thalamic and cortical cells (for review, see Steriade et al., 1993, 1994). Rhythmic burst

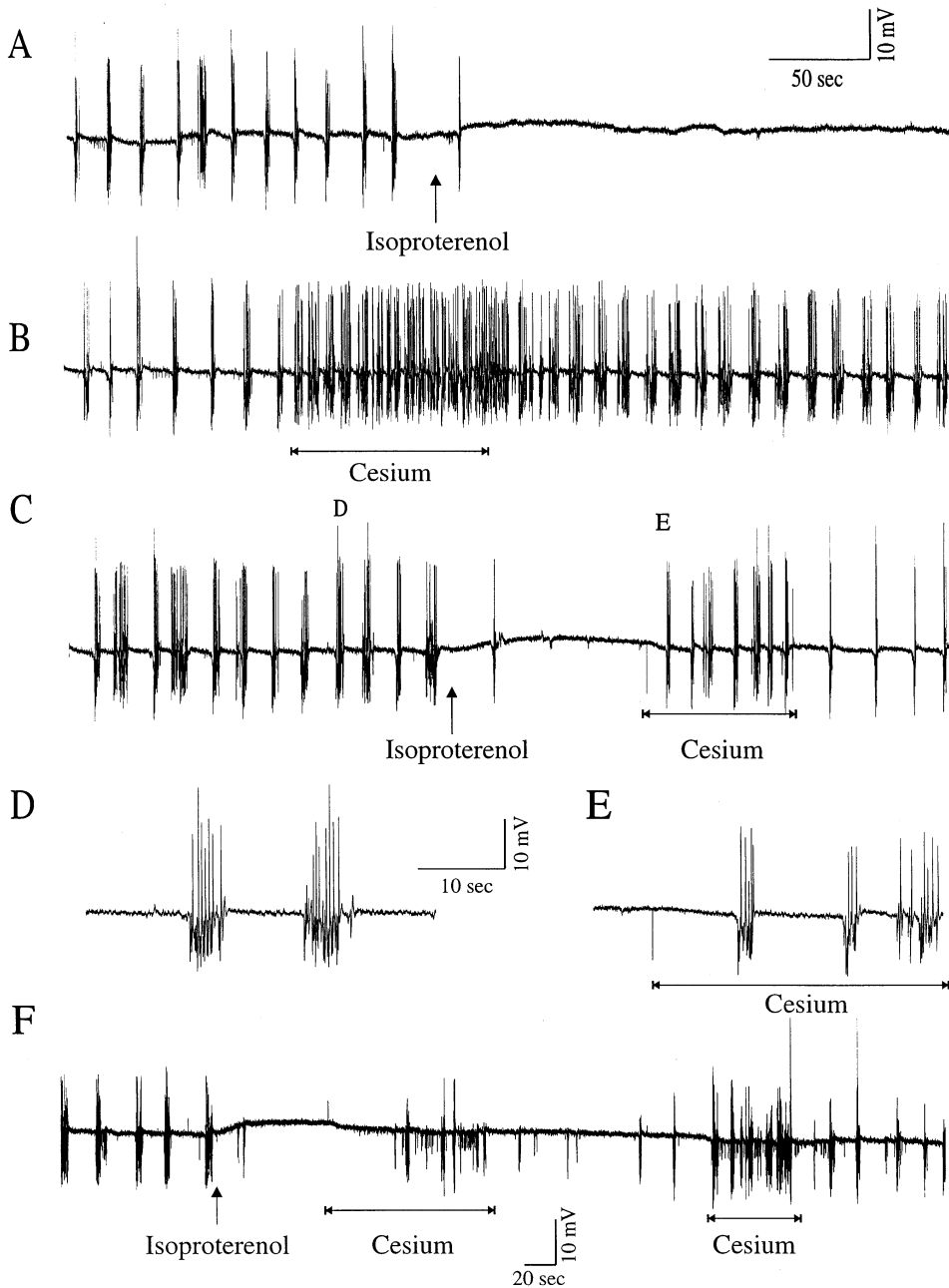


Figure 6. Cesium Reverses the Block of Spindle Oscillations by Isoproterenol

(A) Local application of isoproterenol (50 μ M in micropipette) on the surface of lamina A1 near the intracellularly recorded thalamocortical neuron resulted in a 3 mV depolarization and cessation of spindle oscillations.

(B) Local application of cesium chloride (10 mM in micropipette) at the same location where isoproterenol was applied in (A) resulted in a small membrane hyperpolarization and an increase in frequency of spindle oscillations until a nearly continuous network oscillation was produced. Washout of cesium chloride slowly returned the frequency of occurrence of spindle waves to predrug level.

(C) As before, local application of isoproterenol resulted in an \sim 3 mV membrane depolarization and cessation of spindle oscillations. During this membrane depolarization, cesium chloride was locally applied, which resulted in a membrane hyperpolarization back to -65 mV and return of spontaneous spindle oscillations.

(D) and (E) Expansion of trace in (C) for detail.

(F) In another thalamocortical neuron, shortly after the local application of isoproterenol, which resulted in a 3 mV membrane depolarization and cessation of spindle oscillations, local application of cesium chloride resulted in return of spindle waves. The spindle waves ceased to occur when cesium was discontinued to be locally applied, but then returned upon reapplication of cesium chloride.

firing in single thalamocortical cells is generated largely through the interaction between the low-threshold Ca^{2+} current I_T and the hyperpolarization-activated cation

conductance I_h (McCormick and Pape, 1990a; Soltesz et al., 1991; McCormick and Huguenard, 1992) and has also been observed in vivo (Steriade et al., 1991). The

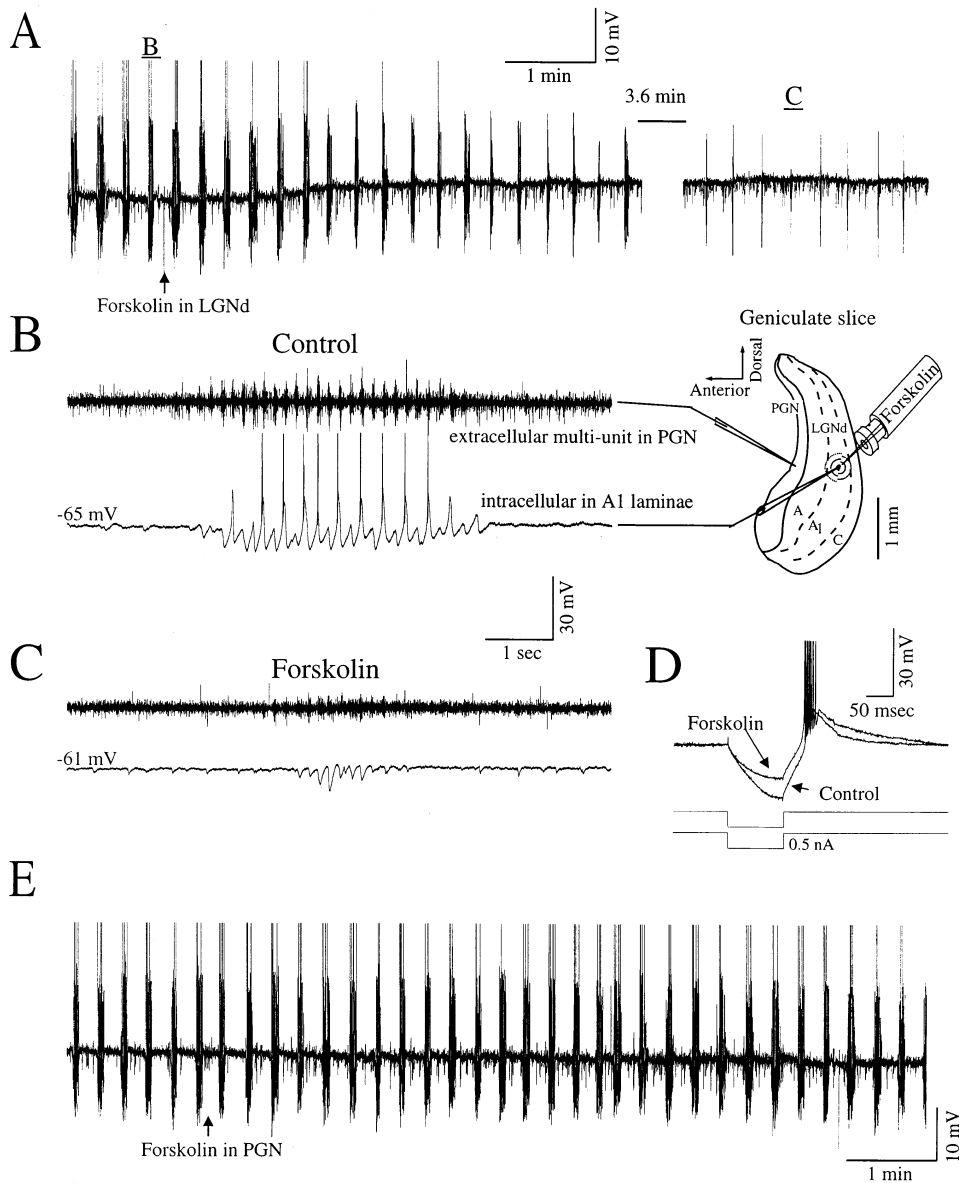


Figure 7. The Adenylyl Cyclase Activator, Forskolin, Decreases the Amplitude and Duration of Spindle Waves in Thalamocortical Neurons but Has No Effect in the PGN

(A) Local application of forskolin ($500 \mu\text{M}$) to the surface of the slice near the intracellularly recorded thalamocortical neuron in lamina A1 resulted in a slow membrane depolarization and markedly reduced the amplitude and duration of spindle waves.

(B) Expansion of one spindle wave before the application of forskolin.

(C) Expansion of the indicated portion of recording in (A) illustrating the marked reduction in the spindle wave IPSPs in the thalamocortical neuron and the multiple unit action potential bursts in the PGN cells.

(D) Examination of the electrotonic membrane response to 100 ms duration, 0.5 nA hyperpolarizing current pulses revealed that forskolin application resulted in an increase in apparent input conductance in the thalamocortical neuron.

(E) Local application of forskolin to the PGN resulted in no change in the characteristics of the spindle waves.

h-current is a mixed $\text{Na}^+\text{-K}^+$ inward current that slowly activates when thalamocortical cells are hyperpolarized to potentials negative to -55 mV and appears to be the generator of the “pacemaker” potential that occurs in between low-threshold Ca^{2+} spikes during rhythmic burst firing in single thalamocortical neurons (McCormick and Pape, 1990a; Soltesz et al., 1991). Experimental studies and computational models of single thalamocortical neurons suggest that the ability of single

thalamocortical cells to generate intrinsic rhythmic bursts of action potentials at 0.5 Hz–4 Hz depends upon the amplitude and properties of the ionic currents involved. For example, activation of β -adrenoceptors or serotonergic receptors on single thalamocortical neurons results in both an enhancement of I_h , through a shift in the voltage dependence of activation to more positive levels, and an abolition of rhythmic burst firing (McCormick and Pape, 1990b). Computational models

of this result suggest that positive shifts in the voltage dependence of I_h will increase the frequency of rhythmic burst firing in the range of 0.5 Hz–4 Hz, but will also decrease the amplitude of each low-threshold Ca^{2+} spike and burst response (McCormick and Huguenard, 1992). Following positive shifts of 5 mV–10 mV, spontaneous rhythmic burst firing may be abolished altogether (McCormick and Huguenard, 1992). Interestingly, reducing I_h below a critical level, either in computational models or with the extracellular application of Cs^+ in vitro, can also block rhythmic burst firing or spindle wave generation, owing to the hyperpolarization of thalamocortical neurons below threshold for generation of low-threshold Ca^{2+} spikes and the block of depolarizing pacemaker potentials (McCormick and Huguenard, 1992; T. Bal, K. H. L., and D. A. M., unpublished data).

Synchronized oscillations in thalamocortical systems during slow-wave sleep are typified by the generation of spindle waves (see Steriade et al., 1993). Spindle waves are 1 s–3 s epochs of synchronized 7 Hz–14 Hz oscillations that are generated as an interaction between thalamocortical and thalamic reticular/PGN neurons (Steriade and Deschênes, 1984; Steriade et al., 1993; von Krosigk et al., 1993; Bal et al., 1995a, 1995b). The generation of a burst of action potentials in the GABAergic neurons of the thalamic reticular/perigeniculate nuclei results in an IPSP that is characterized by a 2 mV–10 mV amplitude hyperpolarization in thalamocortical neurons (Bal et al., 1995a, 1995b). A subset of thalamocortical cells generates a rebound low-threshold Ca^{2+} spike and burst of action potentials, which then returns as a barrage of EPSPs to the thalamic reticular/PGN neurons (Bal et al., 1995a, 1995b). This barrage of EPSPs activates a low-threshold Ca^{2+} spike in the PGN cells, thus initiating the next cycle of the spindle wave. Spindle waves “wax,” or generalize through the progressive recruitment of neurons into this oscillation, presumably owing to axonal interconnections between the thalamocortical and perigeniculate nuclei (Kim et al., 1995). Recently, we have demonstrated that spindle waves “wane,” or progressively diminish, at least in part through the persistent activation of the hyperpolarization-activated cation current I_h (Bal and McCormick, 1995, Soc. Neurosci., abstract, 1996). Thus, we have proposed that the repetitive hyperpolarization of thalamocortical neurons, or the repetitive generation of low-threshold Ca^{2+} spikes, during the generation of spindle waves may result in the persistent activation of I_h , resulting in an abolition of the spindle wave through a reduction in the amplitude of the IPSPs, as well as in their ability to generate rebound low-threshold Ca^{2+} spikes (Bal and McCormick, 1995, Soc. Neurosci., abstract, 1996).

Both spindle waves and intrinsic rhythmic burst discharges disappear in the transition from slow-wave sleep to waking (Andersen and Andersson, 1968; Steriade and Deschênes, 1984; Steriade et al., 1993). This abolition of rhythmic burst firing in thalamocortical circuits is associated with a depolarization of thalamocortical and thalamic reticular neurons from the burst firing to the single-spike mode of action potential generation (Hirsch et al., 1983; Steriade et al., 1986), which has been proposed to result from the release of several

different neurotransmitters, including NE, 5-HT, acetylcholine, histamine, and glutamate (for review, see McCormick, 1992a).

Application of NE to thalamocortical neurons has been demonstrated to result in two direct postsynaptic responses. In addition to the above-mentioned enhancement of I_h through the activation of β -adrenoceptors, activation of α_1 -adrenoceptors results in a decrease in a relatively linear K^+ current termed I_{KL} (McCormick and Prince, 1988; McCormick and Pape, 1990b). Application of 5-HT, on the other hand, to thalamocortical neurons results only in the enhancement of I_h (McCormick and Pape, 1990b). The reduction of I_{KL} by NE results in depolarization of the membrane potential by up to 20 mV, a decrease in apparent input conductance, the abolition of low-threshold Ca^{2+} spikes, and the promotion of single-spike activity (McCormick and Prince, 1988; McCormick, 1992a, 1992b). The functional effects of positive shifts in the activation of I_h are more difficult to demonstrate, although preliminary results suggest that these may be anti-oscillatory through a prevention of the phasic hyperpolarizations that are required for the generation of low-threshold Ca^{2+} spikes (McCormick and Pape, 1990b; McCormick and Huguenard, 1992).

In contrast with thalamocortical cells, application of NE and 5-HT to thalamic reticular/PGN neurons results in a membrane depolarization through the reduction of I_{KL} through the activation of α_1 -adrenoceptors and 5-HT_{2/1C} receptors (McCormick and Wang, 1991). The hyperpolarization-activated cation current I_h is not prominent in thalamic reticular/PGN cells, nor is there evidence for an enhancement of this current by NE or 5-HT in these neurons (McCormick and Wang, 1991).

Considered together with prior results on the activity and release of neuromodulatory transmitters (reviewed by McCormick, 1992a), these studies suggest that the increased release of NE and 5-HT associated with the transition from slow-wave sleep to waking may abolish spindle wave activity both through a decrease in I_{KL} and an enhancement of I_h in thalamocortical neurons and a decrease in I_{KL} in thalamic reticular/PGN cells. Our present results confirm and extend this hypothesis.

First, we demonstrated that depolarization of either thalamocortical or PGN neurons results in an abolition of rhythmic burst firing in both of these cell types during the generation of spindle waves. Although this result is as expected in thalamocortical cells, owing to the lack of rebound low-threshold Ca^{2+} spikes following the phasic IPSPs arriving during spindle wave generation, the finding that this is also true for PGN cells requires elaboration. The barrages of EPSPs that arrive in PGN neurons during the generation of spindle waves are intermixed with IPSPs arising from burst firing in neighboring PGN cells, and these IPSPs dampen the response of the PGN neuron to the EPSP barrage (Bal et al., 1995b). Apparently, the sum of these EPSPs and IPSPs is large enough at membrane potentials of –63 mV to –68 mV to activate low-threshold Ca^{2+} spikes, but are not large enough to increase strongly the pattern of action potential generation when the PGN cell is depolarized into the single-spike firing mode (see Figure 1). Prior dual intracellular recordings from monosynaptically connected PGN and thalamocortical cells have demonstrated that the PGN

cell must generate a high-frequency (350 Hz–450 Hz) burst of action potentials in order to generate a large enough IPSP to generate a rebound burst of action potentials in single thalamocortical neurons (Bal and McCormick, 1995, Soc. Neurosci., abstract, 1996). Together, these results suggest that depolarization of PGN cells should potentially inhibit the generation of spindle waves in thalamic circuits. Indeed, we have found that the depolarization of PGN neurons with the application of NE or 5-HT can result in a complete block of spindle wave generation.

Similarly, depolarization of thalamocortical neurons should also be capable of abolishing spindle wave generation owing to the inhibition of rebound responses that are required for driving PGN neurons to discharge in synchrony. Our present results support this hypothesis in that application of NE in normal bathing medium resulted in a marked depolarization of thalamocortical cells and an abolition of spindle wave generation, although a more thorough investigation of this phenomenon is required.

Our results also support the hypothesis that positive shifts in the voltage dependence of I_h in thalamocortical neurons may abolish the generation of spindle waves. The application to lamina A1 of either 5-HT, the β -adrenoceptor agonist isoproterenol, or the adenylyl cyclase activator forskolin, all resulted in a 1 mV–3 mV depolarization of the membrane potential, an increase in apparent input conductance, and an abolition or marked reduction in the generation of spindle waves. The ability of the local extracellular application of Cs^+ to reverse these effects of β -adrenoceptor stimulation suggests that the abolition of spindle waves is due to the enhancement of I_h , as opposed to some other action of these agents.

Recent *in vitro* studies on the actions of adenosine have revealed that this putative neurotransmitter has postsynaptic actions that are largely opposite to those of NE. Activation of A1 receptors results in both an increase in membrane conductance to K^+ ions and a shift in the voltage dependence of I_h to more negative membrane potentials (Pape, 1992). These results led Pape (1992) to propose that adenosine may have a pro-oscillatory effect in thalamic circuits. However, the bath application of adenosine to rodent thalamic slices results in a reduction in reverberatory interaction between the thalamic reticular nucleus and thalamocortical neurons (Ulrich and Huguenard, 1995). These authors suggest that these anti-oscillatory effects may be mediated through the inhibition of GABA and glutamate neurotransmitter release through the activation of presynaptic purinergic receptors (Ulrich and Huguenard, 1995). The possibility that spindle waves were abolished in our experiments from the inhibition of transmitter release by NE or 5-HT remains to be explored. However, the findings that three different manipulations that enhance I_h all abolish spindle wave generation while the one manipulation that blocks the enhancement of I_h (Cs^+ application) results in the reversal of this abolition all support the hypothesis that the postsynaptic enhancement of I_h is capable of abolishing the generation of spindle waves.

Together with previous results (reviewed by McCormick, 1992), these findings confirm the hypothesis that

the release of NE, 5-HT, and other neurotransmitters may abolish slow-wave sleep-related activity in thalamocortical systems and promote the occurrence of single-spike activity, both through depolarization of the membrane potential through decreases in I_{KL} as well as through the antagonism of hyperpolarizations through the enhancement of I_h . The convergence of multiple neurotransmitters and their postsynaptic responses in the thalamus, including the actions of acetylcholine, NE, 5-HT, glutamate, adenosine, histamine, nitric oxide, and various peptides (reviewed by McCormick, 1992a; Cox et al., 1995; Pape, 1992; Pape and Mager, 1992), suggests that thalamocortical activity may be modulated in a manner that is considerably more complex than merely the depolarization of neurons in the transition from slow-wave sleep to waking. Revealing the subtleties in these neurotransmitter actions and interactions and their functional effects is a difficult but worthwhile task.

Experimental Procedures

For the preparation of slices, male or female ferrets, 3 months old to 4 months old, were deeply anesthetized with sodium pentobarbital (30 mg/kg–40 mg/kg) and killed by decapitation. The forebrain was rapidly removed and the hemispheres were separated with a midline incision. We cut 400 micron-thick slices using a vibratome (Ted Pella, Incorporated) in the sagittal plane. A modification of the technique developed by Aghajanian and Rasmussen (1989) was used to increase tissue viability. During preparation of slices, the tissue was placed in a solution (5°C) in which NaCl was replaced with sucrose while maintaining an osmolarity of 307 mOsm. After preparation, slices were placed in an interface-style recording chamber (Fine Sciences Tools), maintained at $34 \pm 1^\circ\text{C}$, and allowed at least 2 hr to recover. The bathing medium contained (in mM): NaCl, 126; KCl, 2.5; MgSO_4 , 1.2; NaH_2PO_4 , 1.25; CaCl_2 , 2; NaHCO_3 , 26; dextrose, 10; and was aerated with 95% O_2 , 5% CO_2 to a final pH of 7.4. For the first 20 min that the thalamic slices were in the recording chamber, the bathing medium contained an equal mixture of the normal NaCl and the sucrose-substituted solutions.

Intracellular recording electrodes were formed on a Sutter Instruments P-80 micropipette puller from medium-walled glass (WPI, 1B100F) and beveled on a Sutter Instruments beveler. Micropipettes were filled with 2 M K-acetate and 2% biocytin for intracellular labeling of recorded neurons. Biocytin-filled neurons were visualized through standard avidin–biotin–horseradish peroxidase reaction visualized with diaminobenzidine (Horikawa and Armstrong, 1988). In addition, the location of the PGN was confirmed through immunocytochemical staining for GABA (Schwartz and Meineke, 1992). Only those neurons exhibiting a stable resting membrane potential of at least -60 mV and electrophysiological properties as reported previously (Bal and McCormick 1993; McCormick and Pape, 1990a) were included for analysis. Extracellular multiple unit recordings were obtained with tungsten microelectrodes (Frederick Haer, Incorporated).

Drugs were either applied by the pressure–pulse technique in that a brief (10 ms–20 ms; 207 kPa–345 kPa; 10 psi–30 psi) pulse of nitrogen was applied to a broken microelectrode (tip diameter, 2 μm –5 μm) containing the drug dissolved in bathing medium or through addition to the bathing medium. With the pressure–pulse technique, the volume of the resulting application was between 5 pl–15 pl as estimated from the diameter (10 μm –15 μm) of the ejected droplet. For agonists and antagonists, application to the exposed surface of the slice was usually sufficient to elicit its pharmacological effect. Typically, the droplet of drug was formed on the tip of the drug-applying electrode just above the surface of the slice. The micropipette was then lowered to the slice within 50 μm of the entry point of the recording electrode in order to apply the drug. Specific applications to either the LGNd or PGN were obtained by applying the agonists in the LGNd in only lamina A1, ~ 0.5 mm or more from the PGN and by applying the agonists to the anterior

regions of the PGN, at least 150 μm –250 μm from the anterior edge of the LGNd. That the applications were specific is evidenced, for example, by the lack of effect on spindle wave generation by the application of isoproterenol in the PGN, which when applied to the LGNd results in a potent block of spindle wave generation. Both the PGN and the laminae of the LGNd were readily visible with epillumination. In each case where an agonist consistently failed to generate an observable effect in the PGN, subsequent application of the same to lamina A1 was found to have its full effects, indicating that the drug application technique was working. Drugs were obtained from Sigma. The data was analyzed using Axotape (Axon Instruments) on a PC-AT style computer and figures were drawn using CorelDRAW 3.0.

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