

MODULATION OF SPINDLE OSCILLATIONS BY ACETYLCHOLINE, CHOLECYSTOKININ AND 1S,3R-ACPD IN THE FERRET LATERAL GENICULATE AND PERIGENICULATE NUCLEI *IN VITRO*

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Abstract—The transition from sleep to waking is associated with the abolition of spindle waves and the appearance of tonic activity in thalamocortical neurons and thalamic reticular/perigeniculate GABAergic cells. We tested the possibility that changes such as these may arise through modulation of the leak potassium current, I_{KL} , by examining the effects of neurotransmitters known to modulate this current on spindle wave generation in the ferret geniculate slice maintained *in vitro*. Local application of agents that reduce I_{KL} in thalamocortical neurons, including acetylcholine, DL-muscarine chloride and the glutamate metabotropic receptor agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD), to spontaneously spindling thalamocortical neurons resulted in a 5–10 mV membrane depolarization and the abolition of spindle waves. Local application of 1S,3R-ACPD and cholecystokinin-8-sulfate, both of which reduce I_{KL} , to GABAergic neurons of the perigeniculate nucleus resulted in a 10–20 mV membrane depolarization, appearance of tonic discharge and the abolition of spindle wave generation. Local application of 1S,3R-ACPD and cholecystokinin to the perigeniculate nucleus while recording from thalamocortical neurons resulted in the abolition of spindle wave-associated inhibitory postsynaptic potentials and the occurrence of a continuous barrage of smaller amplitude inhibitory postsynaptic potentials, presumably in response to depolarization and tonic discharge of perigeniculate neurons.

These results indicate that modulation of I_{KL} in thalamocortical neurons and perigeniculate neurons is capable of abolishing the generation of spindle waves in thalamic networks. Through the modulation of I_{KL} , ascending and descending activating systems may control the state of the thalamus such that the transition from slow wave sleep to waking is associated with the abolition of slow, synchronized rhythms and the facilitation of a state that is conducive to sensory receptor field analysis, arousal and perception.
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Key words: arousal, sleep, glutamate metabotropic receptor, epilepsy, spindle waves.

The discovery by Moruzzi and Magoun⁵¹ of an ascending brainstem reticular system with “activating” actions on the forebrain was an important step forward in the cellular understanding of sleep/wake mechanisms and in the physiology of states of vigilance. The presence of this reticular activating system was also indicated by the lesion studies of Bremer (reviewed in Ref. 10). In these studies it was observed that a mesencephalic transection caudal to the third nerve was associated with fissured pupils, as in normal non-rapid eye movement sleep, and uninterrupted sequences of waxing and waning electroencephalogram spindle waves. A high spinal transection

at the first cervical level that disconnects the whole encephalon from the spinal cord, in contrast, results in fluctuations between electroencephalogram patterns of both the waking and sleep states. Since these early studies, detailed electrophysiological, neuro-anatomical and pharmacological investigations into the possible neurotransmitter systems that may comprise the ascending reticular activating system have revealed possible roles for acetylcholine (ACh) from the pedunculo-pontine and lateral dorsal tegmental nuclei of the brainstem (and the basal forebrain cholinergic nuclei), norepinephrine (NE) from the locus coeruleus, serotonin (5-HT) from the raphe nuclei, and histamine from the tuberomammillary nucleus of the hypothalamus (reviewed in Refs 47 and 64). In addition, more recent investigations have revealed the possibility of a “descending activating system” from layer VI of the cerebral cortex that may activate glutamate metabotropic receptors⁴⁹ and a peptidergic system which may use cholecystokinin (CCK) as a neurotransmitter.¹⁸

The activation of these neuromodulatory transmitter systems in the thalamus during awakening has

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Abbreviations: ACh, acetylcholine; 1S,3R-ACPD, 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid; CCK, cholecystokinin; CCK-8S, cholecystokinin-8-sulfate; DMPP, 1,1-dimethyl-4-phenylpiperazinium; EPSP, excitatory postsynaptic potential; 5-HT, serotonin (5-hydroxytryptamine); I_{KL} , “leak” potassium current; IPSP, inhibitory postsynaptic potential; LGNd, dorsal lateral geniculate nucleus; NE, norepinephrine; PGN, perigeniculate nucleus.

been proposed to block slow waves (such as spindle waves, delta waves and absence seizures) and induce fast rhythms, and in so doing, enhance the responsiveness of thalamic neurons to prethalamic inputs and consequently the transmission of sensory information (for reviews see Refs 47 and 64). Indeed, the transition from synchronized, rhythmic thalamocortical activity during slow wave sleep to desynchronized, high-frequency activity during arousal and attentiveness is associated with a general increase in the firing rate of cholinergic, noradrenergic, serotonergic and histaminergic neurons.^{2,22,32,56,66,67} The switch in firing mode of thalamic neurons is associated with a depolarization of the membrane potential in thalamocortical cells²⁵ and presumably also in the GABAergic neurons of the perigeniculate nucleus (PGN).^{7,61} Thus, by moving the membrane potential out of the burst firing range towards the range of single-spike firing in both thalamocortical and GABAergic thalamic reticular/PGN neurons, the thalamic network may greatly enhance the transfer of sensory information.^{9,16,37,61,64}

Investigations into the postsynaptic actions of putative modulatory neurotransmitters in the thalamus have revealed that they have in common the ability to control the activities of thalamic neurons, in part, through the reduction of the resting "leak" potassium current, I_{KL} (see Ref. 47).^{18,39,40,45} Activation of muscarinic and glutamate metabotropic receptors on thalamocortical cells results in a reduction of I_{KL} , thereby resulting in a membrane depolarization of 5–20 mV.^{40,48,49} This reduction of I_{KL} results from the activation of a non-pertussis toxin-sensitive G-protein, at least for muscarinic and α_1 -adrenergic receptors,^{40,41} and functionally it is associated with the abolition of rhythmic burst firing in single thalamocortical neurons and the promotion of single-spike activity. Based upon this and similar findings, it has been hypothesized that the neuromodulation of I_{KL} may underlie the transition from rhythmic, synchronized oscillations associated with slow wave sleep to the tonic, single spike activity associated with waking (reviewed in Ref. 47).

To test this hypothesis, we examined the effects of neurotransmitters that are known to modulate the activities of I_{KL} on spindle wave generation in spontaneously spindling ferret dorsal lateral geniculate nucleus (LGNd) slices maintained *in vitro*.^{4,5} Previous investigations in this preparation have demonstrated that spindle waves, similar to those associated with slow wave sleep,⁶⁵ are generated through an interaction between the GABAergic neurons of the PGN and the thalamocortical cells of the LGNd.^{4,5,69} Portions of the results have appeared in abstract form.^{33,34}

EXPERIMENTAL PROCEDURES

For the preparation of slices, male or female ferrets (*Mustela putorius furo*; Marshall Farms, North Rose, NY, U.S.A.), three to four months old, were deeply anesthetized

with sodium pentobarbital (30–40 mg/kg) and killed by decapitation. The forebrain was rapidly removed and the hemispheres were separated with a midline incision. Four-hundred-micrometer-thick slices were cut using a Vibratome (Ted Pella, Inc.) in the sagittal plane. A modification of the technique developed by Aghajanian and Rasmussen¹ 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 $35 \pm 1^\circ\text{C}$ and allowed at least 2 h 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% $\text{O}_2/5\% \text{CO}_2$ 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 pulled from medium-walled glass (WPI, 1B100F) and beveled on a Sutter Instruments beveler. Micropipettes were filled with 2 M potassium 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.²⁷ In addition, the location of the PGN was confirmed through immunocytochemical staining for GABA.⁵⁸ Only those neurons exhibiting a stable resting membrane potential of at least -60 mV and electrophysiological properties as reported previously^{3,42} were included for analysis.

Drugs were either applied by the pressure-pulse technique, in which a brief (10–20 ms; 207–345 kPa; 10–30 psi) pulse of nitrogen was applied to a broken microelectrode (tip diameter 2–5 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 and 15 pl as estimated from the diameter of the ejected droplet. Application of drugs to the exposed surface of the slice was usually sufficient to elicit its pharmacological effect. However, in the case of ACh, it was necessary to lower the drug-applying pipette into the slice to obtain robust responses. Typically, the entry point of the drug-applying electrode was within 50 μm of the recording electrode. Although it is not possible to estimate accurately the effective concentration of applied agonists at the receptors of the recorded neuron, previous investigations have suggested that it is at least 10-fold less than that contained in the micropipette.⁴⁵ 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) and cholecystokinin-8-sulfate (CCK-8S) were obtained from RBI. All other drugs were obtained from Sigma. The data were analysed using Axotape (Axon Instruments) on a PC-AT style computer and figures were drawn using Corel Draw 3.0.

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 Fig. 1). As reported previously,⁵ during the generation of spindle waves LGNd thalamocortical neurons received barrages of inhibitory postsynaptic potentials (IPSPs) at a frequency of 6–10 Hz and these IPSPs resulted in the generation of rebound low-threshold Ca^{2+} spikes (see Fig. 1). Simultaneous extracellular multiple unit and

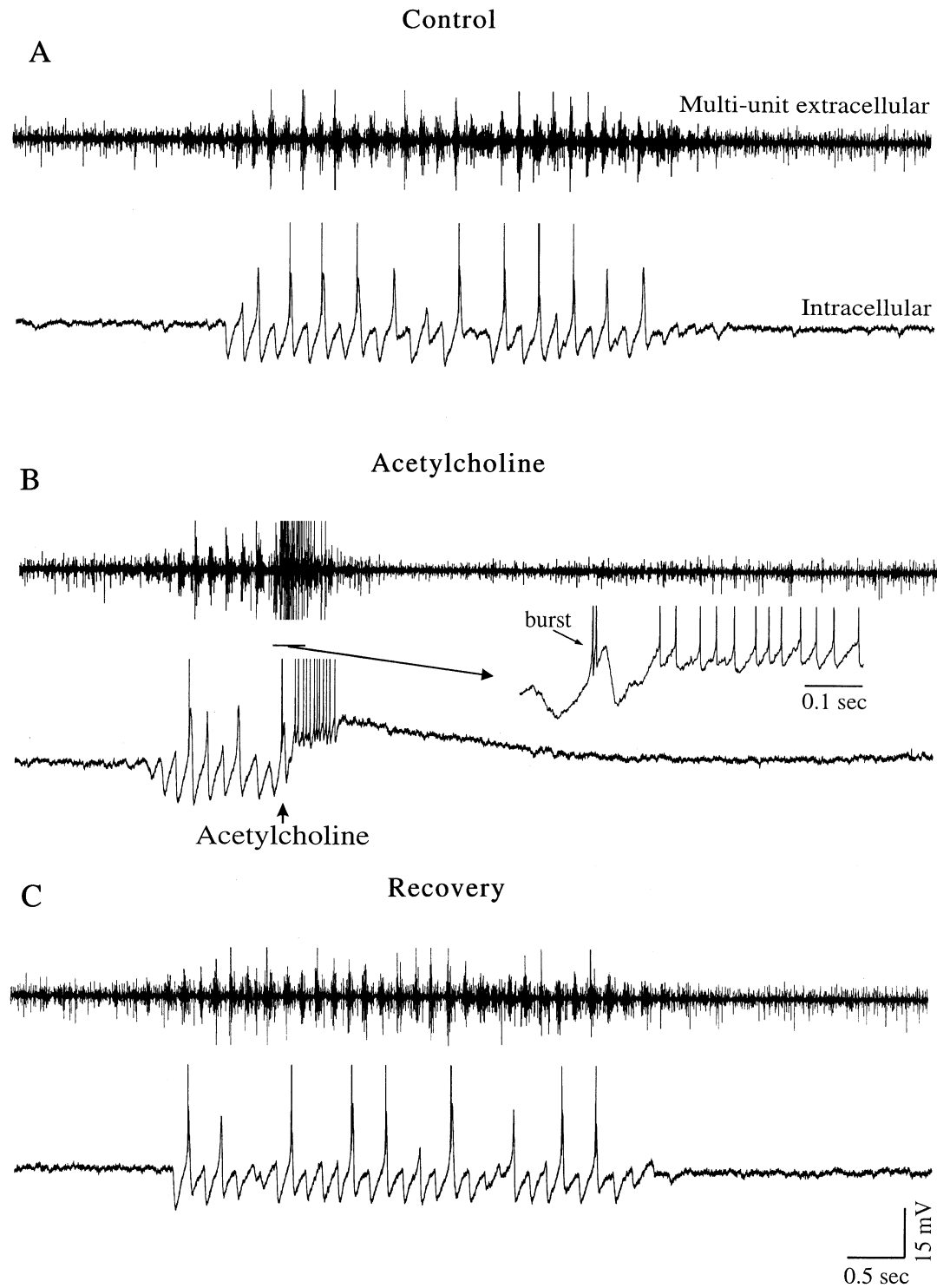


Fig. 1. Intracellular and multi-unit extracellular recordings of response to ACh in spontaneously spindling neurons of lamina A1. (A) A single spindle wave demonstrating the synchronization between the intracellularly recorded thalamocortical cells and the nearby extracellularly recorded neurons. The two electrodes were within 50 μm of each other. (B) Local application of ACh (5 mM in micropipette) near the recorded cells resulted in the rapid inhibition of a spindle wave associated with the block of the IPSPs arriving in this cell, as well as a membrane depolarization and tonic firing. (C) These effects of ACh are completely reversible. Additional information is available at <http://info.med.yale.edu/neurobiol/mccormick/mccormick.html>.

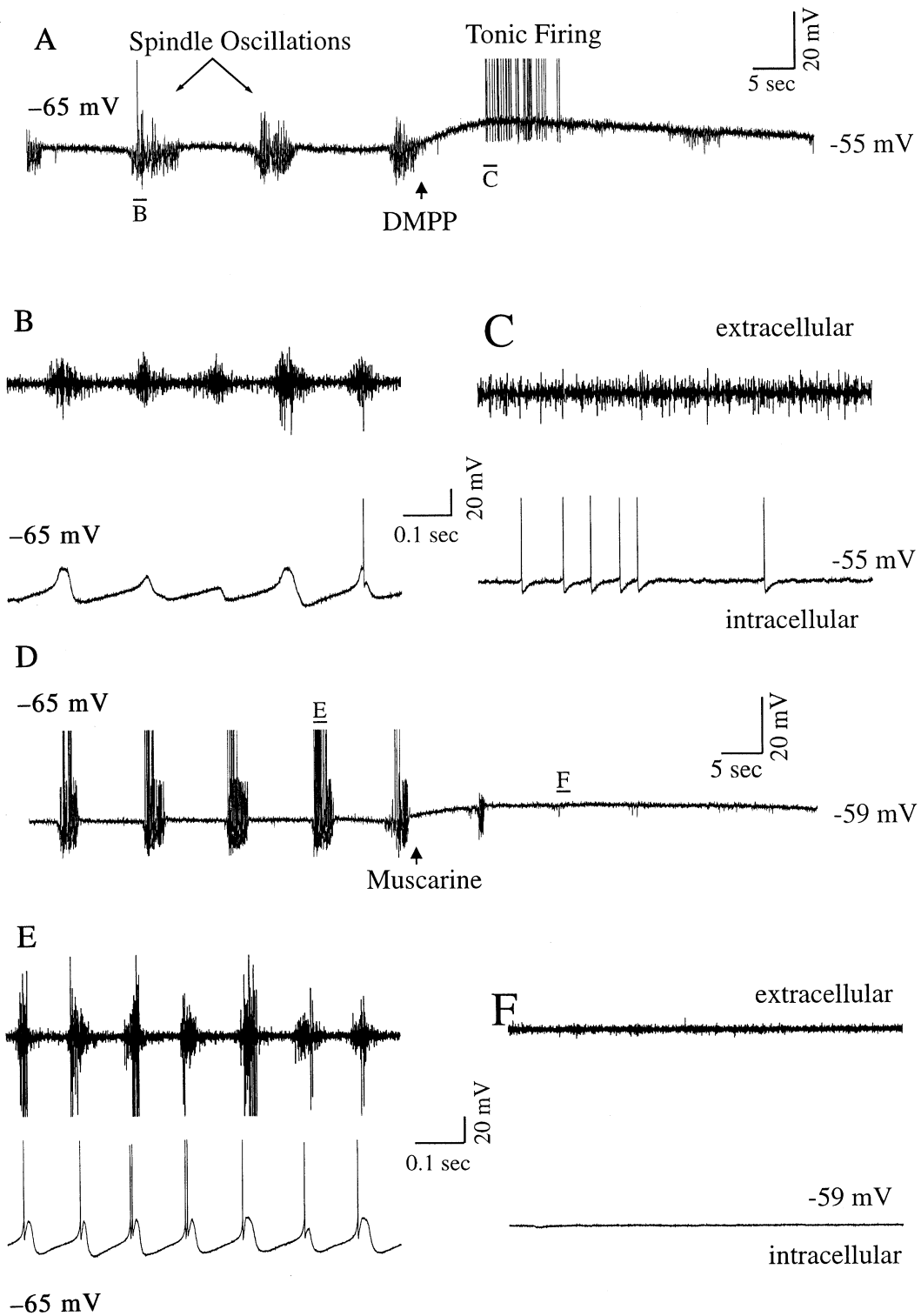


Fig. 2. Effects of activation of nicotinic and muscarinic receptors on the generation of spindle waves in the LGNd. (A) Local application of the nicotinic receptor agonist DMPP (1.0 mM in micropipette) depolarized the thalamocortical neuron, resulting in tonic firing. (B) Expansion of a portion of a spindle oscillation before the application of DMPP. An extracellular electrode was placed within 50 μ m of the intracellular electrode in the A1 laminae. (C) After the application of DMPP, spindle oscillations are blocked and the occurrence of tonic activity is seen. (D) Local application of the muscarinic receptor agonist DL-muscarine chloride (500 μ M in micropipette) depolarized the thalamocortical neuron and stopped spontaneous spindle oscillations. (E, F) Expansion of the indicated portion of recording in D for detail. Application of muscarine depolarized thalamocortical cells but generally did not result in the generation of tonic firing. Following washout of these agonists, spindle waves returned to normal (not shown).

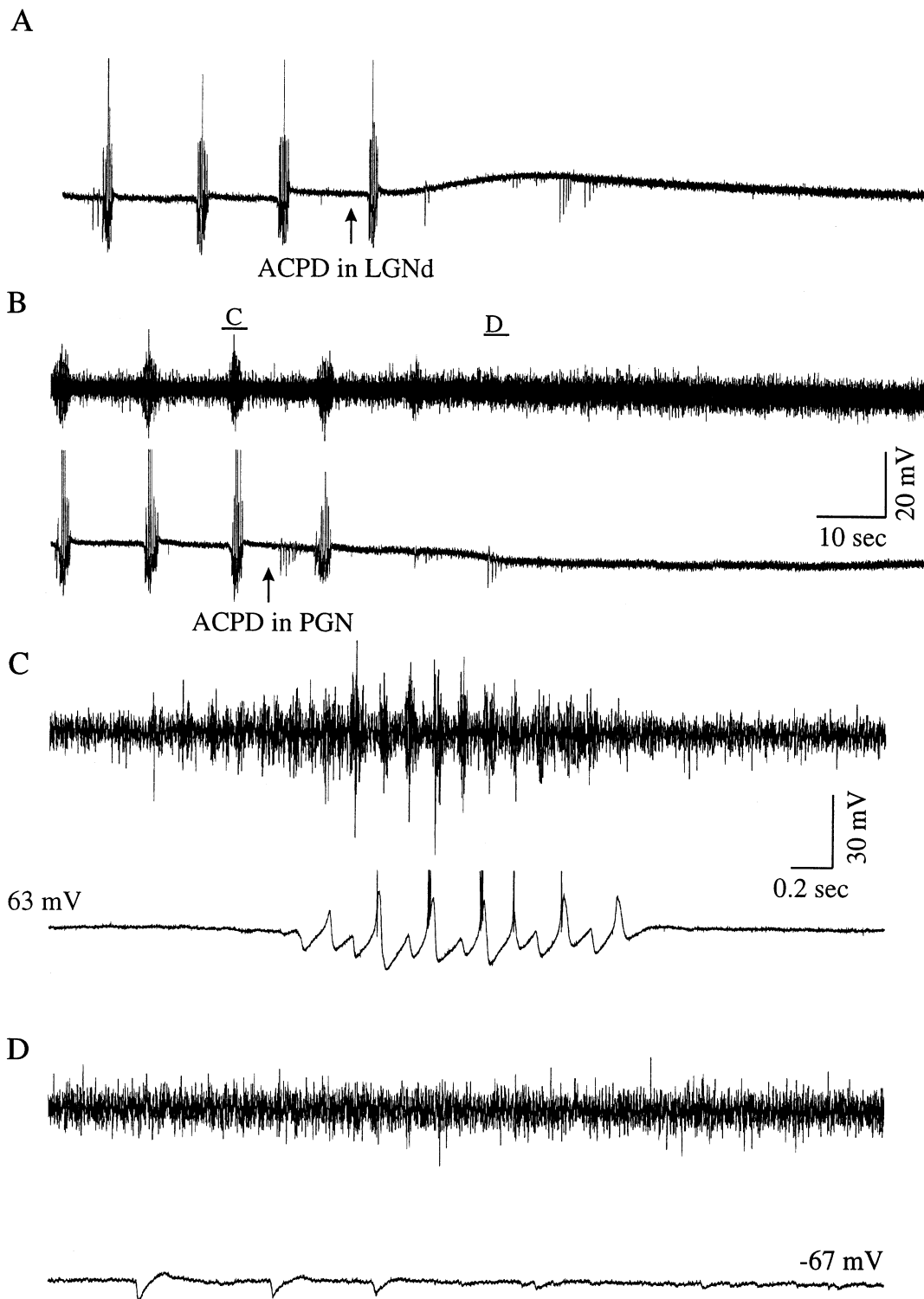
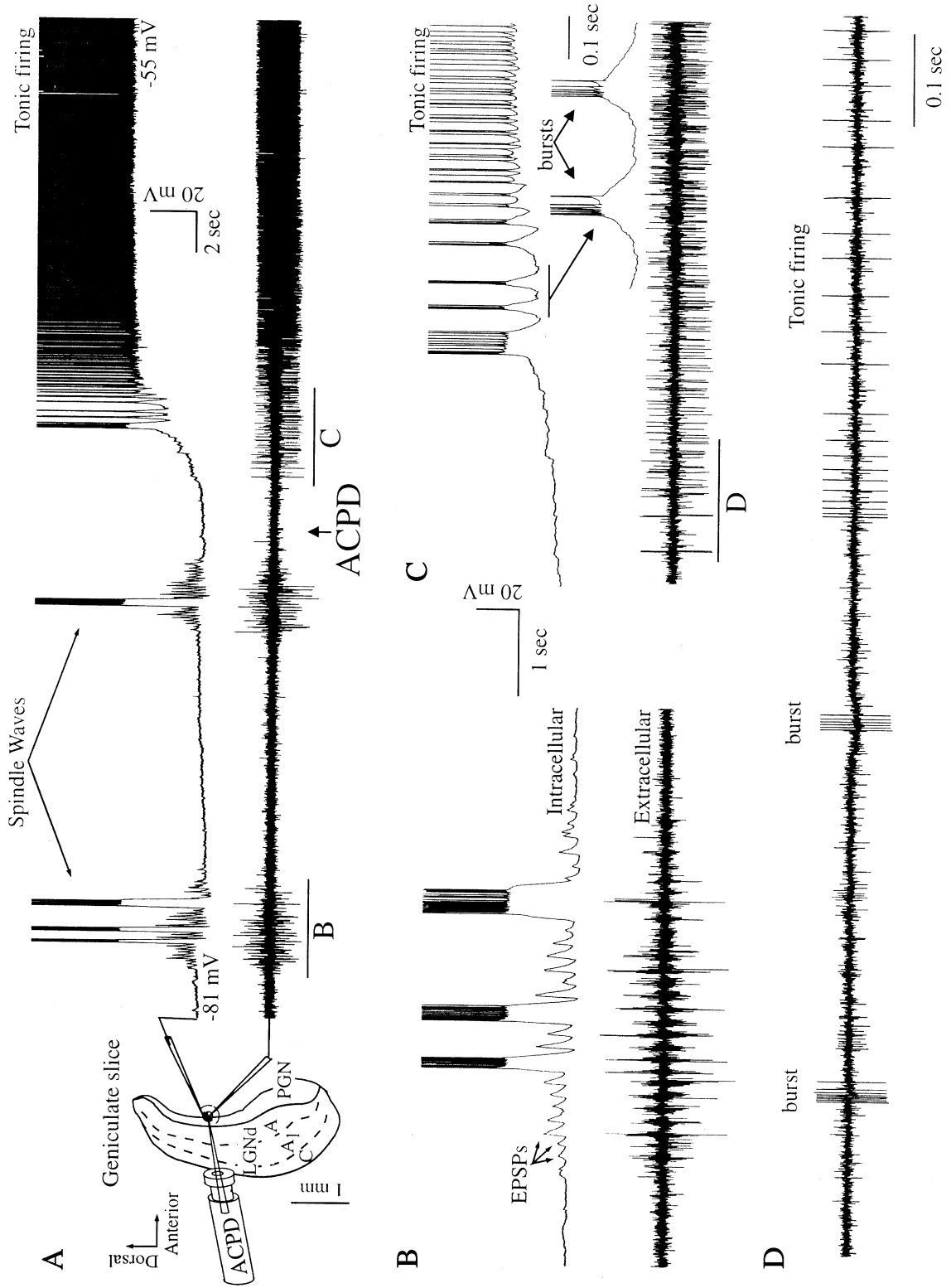


Fig. 3. Application of the glutamate metabotropic receptor agonist 1*S*,3*R*-ACPD results in depolarization of the membrane potential and the cessation of spindle oscillations in an LGNd thalamocortical neuron. (A) Local application of 1*S*,3*R*-ACPD to spontaneously spindling, intracellularly recorded thalamocortical neuron resulted in a 9 mV membrane depolarization and block of spindle oscillations. (B) Local application of 1*S*,3*R*-ACPD to the PGN area immediately anterior to the recording site, where both extracellular and intracellular electrodes were placed within 50 μ m of each other, resulted in 4 mV membrane hyperpolarization (from -63 to -67 mV), appearance of tonic IPSPs and block of spindle oscillations. (C) Expansion of one spontaneous spindle wave illustrating the spindle IPSPs followed by rebound low-threshold Ca^{2+} spikes. (D) Expansion of the point indicated in B illustrates three IPSPs that are presumably due to bursting and a train of smaller IPSPs that are due to tonic firing in PGN neurons that innervate this thalamocortical neuron. Perigeniculate or thalamic reticular cells typically generate a repetitive burst followed by a tonic pattern of action potentials associated with the depolarizing actions of neuromodulatory substances.⁴⁵ Following washout of 1*S*,3*R*-ACPD, spindle waves returned to normal (not shown).



intracellular recordings from thalamocortical cells revealed the synchronization of action potential bursts associated with spindle wave generation (Fig. 1).

Intracellular recordings of the GABAergic neurons of the PGN revealed two distinct modes of action potential generation: rhythmic burst firing at rest and tonic single-spike activity at more depolarized levels. As reported previously,⁴ during the generation of a spindle wave these neurons generated repetitive (2–9 Hz) high-frequency (up to 500 Hz) burst discharges mediated by the activation of a low-threshold Ca^{2+} spike by the arrival of barrages of excitatory postsynaptic potentials (EPSPs), presumably arising from the LGNd thalamocortical neurons (e.g., see Fig. 4).

Previous studies have demonstrated that, in the thalamocortical neurons, a decrease in membrane potassium conductance, termed $I_{\text{K,L}}$, occurs through the activation of muscarinic and glutamate metabotropic receptors.^{40,41,49} Similarly, in the GABAergic thalamic reticular/PGN neurons, a decrease in $I_{\text{K,L}}$ may also occur through the activation of glutamate metabotropic and CCK receptors.^{3,18,45} Here, we examined how activation of these receptors affects spontaneous spindle wave generation in thalamocortical and GABAergic PGN neurons.

Acetylcholine

Intracellular recording from a thalamocortical neuron in lamina A1 revealed spontaneous spindle wave generation characterized by repetitive barrages of IPSPs followed by rebound Ca^{2+} burst sequences. These action potential bursts were synchronized with the action potential activities of neighboring neurons as recorded by a multi-unit extracellular electrode placed within 50 μm of the intracellular electrode (Fig. 1A). During the middle of one spindle sequence, application of ACh (5 mM in micropipette), 50–100 μm from the surface of the slice and within 50 μm of the recording electrodes, resulted in rapid membrane depolarization, block of the spindle wave and the appearance of tonic discharge (Fig. 1B; $n=4$). The onset latency of the depolarization was as brief as the delay imposed by the application system (i.e. 10–30 ms). This effect of ACh was reversible (Fig. 1C).

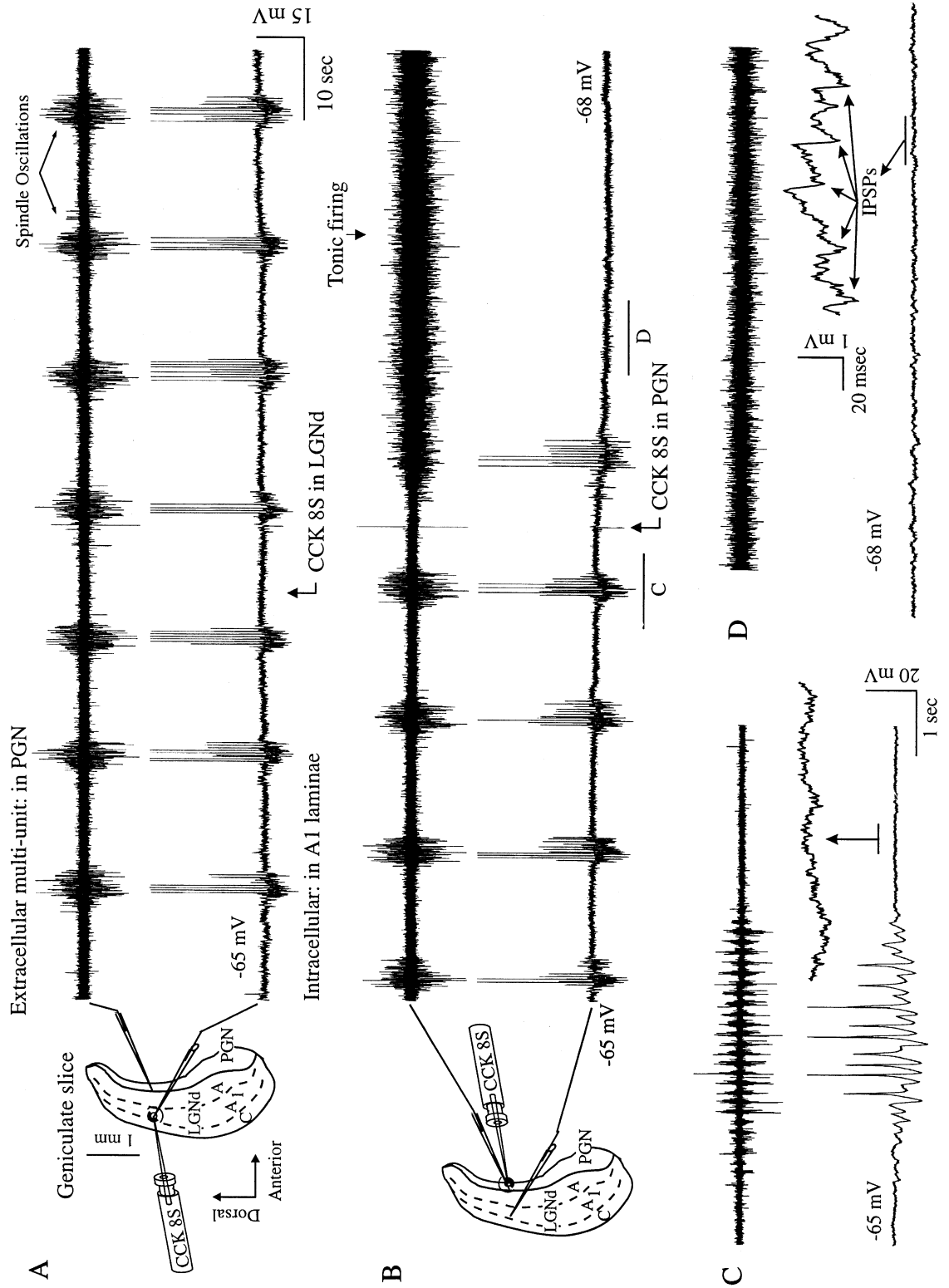
We investigated the pharmacological profile of the receptors mediating the responses of LGNd thalamocortical neurons to ACh, using typical nicotinic and

muscarinic agonists. Application of the nicotinic receptor agonist 1,1-dimethyl-4-phenylpiperazinium (DMPP; 1 mM in micropipette) to the local region of intracellularly recorded thalamocortical neurons resulted in a slow membrane depolarization of approximately 9–10 mV, cessation of spontaneous spindling and the generation of tonic firing (Fig. 2A–C; $n=4$). Similarly, application of the muscarinic receptor agonist DL-muscarine chloride (500 μM in micropipette) also resulted in a slow membrane depolarization of approximately 3–6 mV and the cessation of spontaneous spindling, but did not result in the generation of tonic firing (Fig. 2D–F; $n=5$). These results suggest that the depolarizing and spindle blocking action of ACh may be mediated by the nicotinic as well as the muscarinic receptor⁷⁰ activation. Previous investigations have shown that the rapid depolarizing effects of ACh, such as that in Fig. 1B, are mediated through nicotinic receptors.⁴⁰

Glutamate metabotropic receptors

Similar to muscarinic receptor activation, local application of the glutamate metabotropic receptor agonist 1S,3R-ACPD (0.5 mM in micropipette) to a thalamocortical neuron also resulted in a 5–9 mV membrane depolarization and block of spindle wave generation (Fig. 3A; $n=6$). In addition, application of 1S,3R-ACPD to the PGN while recording extracellularly and intracellularly from thalamocortical neurons in lamina A1 revealed an abolition of spindle wave generation (Fig. 3B; $n=3$). This block of spindle waves was associated with the appearance of a train of three to six large-amplitude IPSPs (2–6 mV) followed by a tonic barrage of many smaller-amplitude IPSPs (0.1–1 mV), which resulted in a prolonged hyperpolarization (1–3 mV) of the membrane potential of the thalamocortical neuron (Fig. 3B, D). This switch in amplitude and frequency distribution of IPSPs arriving in thalamocortical neurons suggested that application of 1S,3R-ACPD to the PGN resulted in a shift in firing mode of these cells.⁴⁵ Indeed, local application of 1S,3R-ACPD to the surface of the slice near simultaneously recorded extracellular multi-unit and intracellular electrodes in the PGN revealed a membrane depolarization of 10–26 mV, the generation of several bursts of high-frequency action potentials followed by tonic discharge, and the abolition of spindle waves (Fig. 4; $n=5$). These results

Fig. 4. Application of the glutamate metabotropic receptor agonist 1S,3R-ACPD results in depolarization of the membrane potential and the cessation of spindle oscillations in GABAergic PGN neurons. (A) Simultaneous extracellular multi-unit and intracellular recording from the PGN within 50 μm of each other. Local application of 1S,3R-ACPD resulted in the appearance of several bursts of action potentials followed by tonic firing, and block of spindle oscillations. This was associated with a large membrane depolarization in the intracellularly recorded GABAergic PGN neuron. (B) Expansion of one spontaneous spindle wave indicated in A, illustrating the spindle EPSPs followed by rebound low-threshold Ca^{2+} spikes. (C) Expansion of the point indicated in A illustrates several bursts and train of action potentials. (D) Expansion of the point indicated in C, illustrating two bursts and tonic firing in an extracellularly recorded PGN neuron. Following washout of CCK, spindle waves returned to normal (not shown).



indicate that glutamate metabotropic receptor activation in both thalamocortical and GABAergic PGN cells is able to depolarize the membrane potential of both of these cell types and block spindle wave generation, presumably through a decrease in I_{KL} .

Cholecystokinin

Simultaneous recordings obtained from an intracellular electrode in a thalamocortical neuron in lamina A1 and extracellular multi-unit electrode placed in the PGN area immediately anterior to the intracellular electrode revealed the generation of synchronous spindle waves. Local application of CCK-8S (Fig. 5A; $n=4$; 100 μ M in micropipette) to lamina A1 did not affect spindle wave generation in either the LGNd or the PGN. However, application of CCK-8S to the PGN resulted in tonic discharges and the abolition of spindle waves in the extracellularly recorded PGN neurons (Fig. 5B; $n=10$). This tonic discharge of PGN neurons was associated with a tonic barrage of many small (<1 mV) IPSPs that together resulted in a prolonged hyperpolarization (1–3 mV) of the membrane potential in thalamocortical cells (Fig. 5B, D).

To test the intracellular effect of CCK on PGN neurons, the recording configuration was switched such that simultaneous recordings were obtained from an intracellular electrode in a GABAergic PGN neuron and an extracellular multi-unit electrode was placed in lamina A1 immediately posterior to the intracellular electrode. As before, local applications of CCK-8S to the surface of the slice in the LGNd did not alter spindle wave generation in either the PGN or the LGNd (Fig. 6A; $n=4$). However, when CCK-8S was applied locally to the surface of the slice in the PGN area, a slow membrane depolarization, appearance of tonic discharge and abolition of spontaneous spindle wave generation was observed (Fig. 6B, D; $n=4$). Spindle wave generation from the presumed thalamocortical neurons in lamina A1 was also blocked during this local application of CCK-8S to the PGN (Fig. 6B). As the depolarizing effect of CCK-8S diminished, spindle waves returned (Fig. 6E). These results indicate that activation of CCK receptors in the perigeniculate, but not in thalamocortical, neurons results in depolarization of the membrane potential, generation of tonic discharge

and blockage of spindle wave generation, presumably through a reduction in I_{KL} .

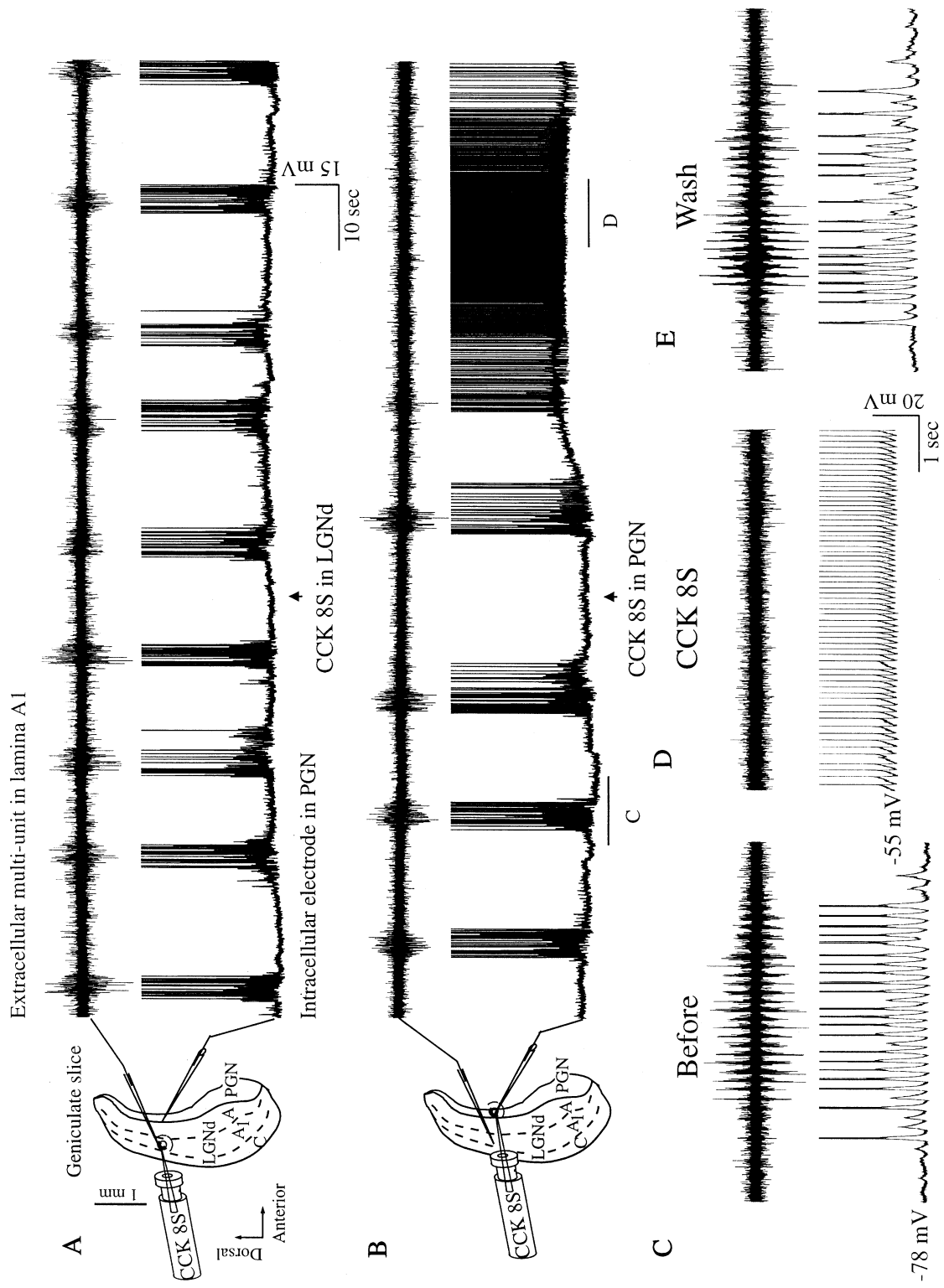
DISCUSSION

The results of the current experiments indicate that the switch in firing modes of thalamocortical neurons and in GABAergic neurons of the PGN in the transition from sleep to waking may involve, in part, the modulation of the leak potassium current I_{KL} by the release of various putative neurotransmitters, including ACh, CCK and perhaps glutamate.

The cholinergic system

The LGNd is densely innervated by cholinergic fibers arising from the pedunculopontine tegmental nucleus and the parabigeminal nucleus,^{19,20} while the thalamic reticular nucleus receives cholinergic projections from both brainstem and basal forebrain nuclei.^{24,59,62,72} Electrical stimulation in the brainstem region of the cholinergic input to the thalamus in anesthetized and reserpine-pretreated cats produces a short-latency depolarization followed by a long-lasting period of hyperpolarization in intracellularly recorded perigeniculate neurons,²⁸ while a transient depolarizing response often interrupted by one or two short-duration IPSPs is seen in thalamocortical neurons.²⁹ Electrical stimulation of the brainstem cholinergic input to the thalamus during the generation of a spindle wave results in the interruption and suppression of the spindle.²⁸ Similarly, we found that application of ACh to the LGNd can interrupt the generation of a spindle wave. Presumably, this rapid depolarizing action of ACh results from the activation of nicotinic receptors, since the activation of muscarinic receptors results in a slow depolarization that takes several seconds to reach peak amplitude (also see Ref. 40). In either case, the activation of nicotinic or muscarinic receptors in the LGNd is capable of suppressing the generation of spindle waves. Presumably, this effect is mediated largely by the depolarization of thalamocortical neurons. Depolarization of these cells results in suppression of rebound low-threshold Ca^{2+} spikes following the arrival of IPSPs from burst firing in PGN cells, owing to inactivation of the low-threshold Ca^{2+} current. The abolition of rebound low-threshold

Fig. 5. CCK depolarizes neurons and blocks spindle waves when applied to the PGN but not when applied to the LGNd. (A) Simultaneous extracellular multi-unit activity in the PGN and intracellular activity in a thalamocortical neuron in lamina A1 of the LGNd. Local application of CCK-8S in lamina A1 was without discernible effect on the generation of spindle waves. (B) Local application of CCK-8S in the PGN resulted in cessation of spindle oscillations and initiation of continuous tonic firing. In the LGNd, the intracellularly recorded thalamocortical neuron was hyperpolarized by 3 mV (from -65 to -68 mV) and the spindle oscillations were abolished. (C) Expansion of one spontaneous spindle wave before the application of CCK-8S. (D) Expansion of the point indicated in B illustrates the repetitive IPSPs in the thalamocortical neuron and the repetitive tonic firing of the GABAergic PGN neurons, as seen in the extracellular recording, following the application of CCK. Following washout of CCK, spindle waves returned to normal (not shown).



Ca²⁺ spikes in thalamocortical neurons removes the phasic activation of the GABAergic PGN neurons, and therefore spindle waves are suppressed.

In contrast to the depolarizing effects of ACh on thalamocortical neurons, application of ACh to PGN cells results in a rapid nicotinic depolarization followed by a muscarinic hyperpolarization.^{35,39} Together, these results suggest that the activation of the ascending cholinergic system may abolish spindle wave generation through the rapid nicotinic depolarization of thalamocortical and thalamic reticular neurons, the following muscarinic inhibition of thalamic reticular cells, and perhaps most importantly, the prolonged depolarization of thalamocortical cells. One interesting possibility is that the phasic activation of nicotinic receptors in thalamocortical and/or thalamic reticular cells may actually initiate the generation of a spindle wave. For example, during slow wave sleep, “K-complexes” are associated with a large sharp wave throughout the scalp recording electrodes followed by the generation of a spindle wave (see Ref. 52). Intracellular recordings of similar events in anesthetized cats reveal that they are associated with rapid depolarization of cortical, thalamocortical and thalamic reticular neurons, followed by the generation of a spindle wave.^{17,65} Although it seems likely that this event may be generated as a consequence of cellular interactions within thalamocortical systems, it is also possible that they are generated in response to the rapid activation of thalamocortical systems by an ascending activating pathway, such as the brainstem cholinergic system. Indeed, previous electrophysiological and pharmacological investigations of another phasic activation in thalamocortical systems, the ponto-geniculo-occipital waves, suggests that they are mediated by the phasic activation of brainstem cholinergic neurons.⁶³ Our working hypothesis is that phasic, intermittent activation of ascending cholinergic systems may result in the rapid and short-duration activation of thalamocortical systems, while more tonic activation of ascending cholinergic neurons, such as during periods of wakefulness and rapid eye movement sleep, will result in the suppression of slow wave sleep rhythms, such as spindle waves, through the tonic depolarization of thalamocortical neurons and cortical pyramidal cells.⁴⁰

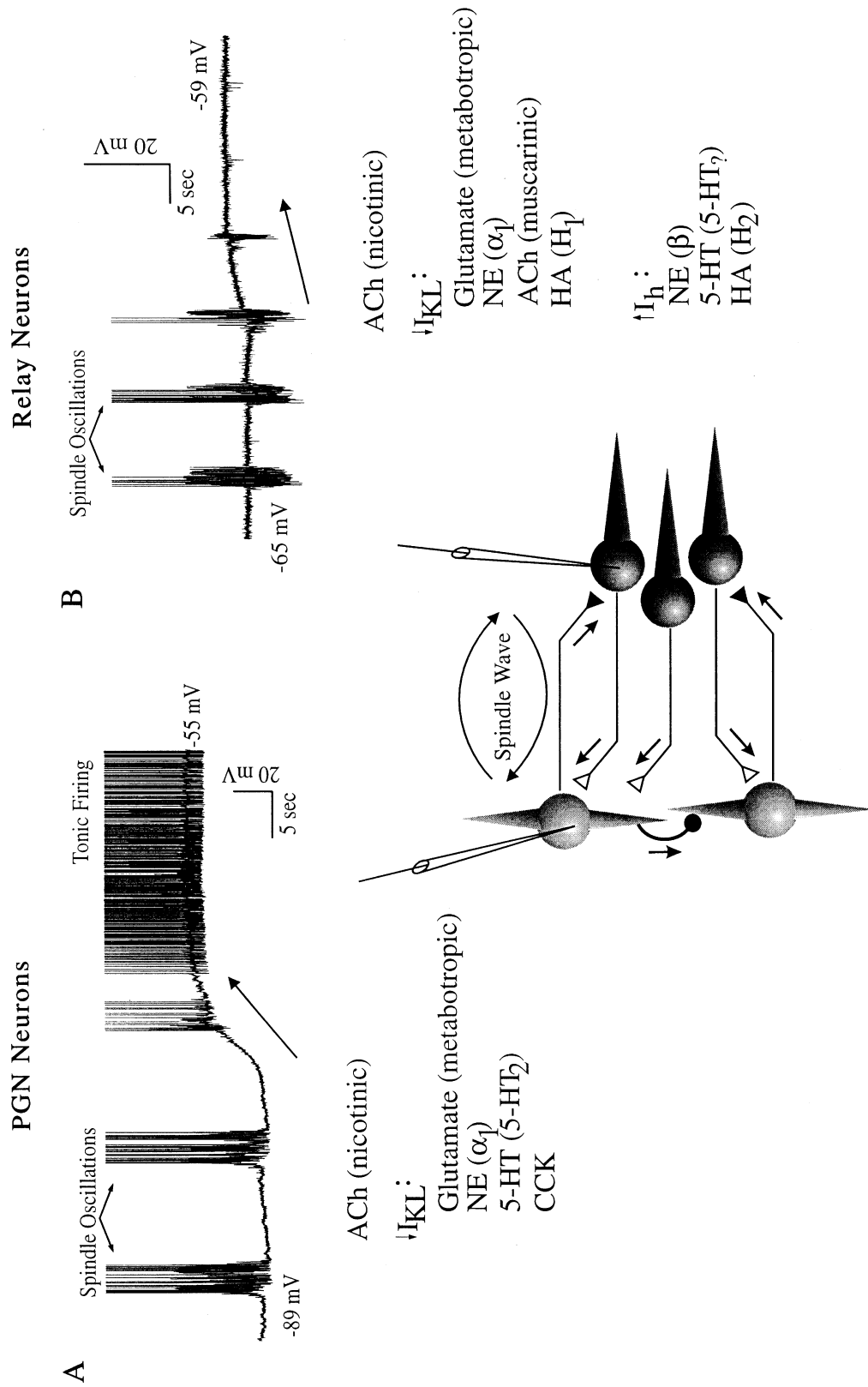
Glutamate metabotropic receptors

In addition to the “ascending” reticular activating system, recent work has suggest the possibility of a “descending” activating system formed by the corticothalamic pathway arising from layer VI of the neocortex.⁴⁹ Anatomically, corticothalamic fibers form the major synaptic input to thalamic relay and GABAergic thalamic reticular/PGN neurons, suggesting that this pathway has an important function in the regulation of thalamocortical activity.^{50,71} Activation of the corticothalamic path results in the generation of rapid EPSPs mediated by *N*-methyl-D-aspartate and non-*N*-methyl-D-aspartate receptors in thalamocortical cells.^{21,26,57,68} Following these fast EPSPs is a slow EPSP that may be up to 10 mV in amplitude and 1 min in duration after just 10 stimuli.⁴⁹ This slow EPSP may be mediated by the activation of glutamate metabotropic receptors, since application of the agonist 1*S*,3*R*-ACPD mimics this event and the maximal stimulation of glutamate metabotropic receptors occludes the corticothalamic slow EPSP.⁴⁹ The effects of glutamate metabotropic receptor antagonists on this slow EPSP remain to be explored.⁴⁹ The present findings that glutamate metabotropic receptor activation in PGN or LGNd thalamocortical neurons by 1*S*,3*R*-ACPD results in membrane depolarization and block of spindle oscillations provide support for the theory that arousal may, in part, be a consequence of increased activity in the thalamocortical and corticothalamic circuit itself.^{13,14,49} An additional possibility that remains to be explored is that brainstem systems that utilize glutamate as a neurotransmitter¹⁵ may also activate glutamate metabotropic receptors and form an additional component to the ascending activating system.

Modulation by cholecystokinin

In situ hybridization histochemistry for the distribution of CCK mRNA has revealed that many neurons (90%) of the rodent LGNd contain high levels of CCK transcripts, while neurons of the thalamic reticular nucleus are apparently devoid of signal.¹¹ Further, CCK is present in corticothalamic neurons and thalamocortical cells,^{12,30} both of which innervate the thalamic reticular nucleus.³¹ Although thalamic reticular neurons do not contain CCK, this

Fig. 6. CCK-8S depolarizes PGN GABAergic neurons. (A) Simultaneous extracellular multi-unit recording in lamina A1 and intracellular recording in the PGN. Local application of CCK-8S in lamina A1 results in no change in activity at either recording site. (B) Local application of CCK-8S to the PGN results in a slow membrane depolarization, appearance of tonic discharge and abolition of spontaneous spindle waves in the intracellularly recorded PGN neuron, while spindle waves were also blocked in the extracellular multi-unit recording in lamina A1. (C) Expansion of one spontaneous spindle oscillation in B before the application of CCK-8S. (D) Expansion of the indicated point in B illustrates the repetitive single spikes occurring in the PGN neuron, while only background activity is seen extracellularly in extracellular multi-unit recording in lamina A1. (E) With washout of CCK-8S, the membrane potential of the PGN neuron re-hyperpolarizes to pretreatment level and spontaneous spindles return in both the PGN and LGNd.



nucleus exhibits a high density of CCK binding sites.^{55,73} It has recently been suggested by Cox *et al.*¹⁸ that CCK may serve as an endogenous modulator whose release will depend on patterns or levels of activity in corticothalamic and thalamocortical cells. Since high-frequency spiking, as may occur during spindle waves, is optimal for peptide release in some systems,⁸ the successive spike bursts in thalamocortical neurons during a spindle wave may lead to the release of CCK on the thalamic reticular neurons. We report here that local application of CCK-8S to the GABAergic neurons of the thalamic reticular/PGN system results in depolarization and block of spindle wave generation. As reported by Cox *et al.*,¹⁸ the application of CCK-8S to thalamocortical cells did not have any discernible affect on these neurons.

Possible scenario of neurotransmitter control of arousal

Thalamocortical neurons and the GABAergic neurons of the thalamic reticular/PGN system both display two distinct modes of action potential generation: rhythmic bursting occurs during drowsiness and slow wave sleep, and tonic single-spike firing occurs during periods of wakefulness and arousal.^{33,60,61} Extracellular recordings from identified thalamocortical³⁸ and thalamic reticular neurons^{7,61} during the transition from slow wave sleep to waking revealed that these cells switch from rhythmic burst firing to the generation of trains of action potentials. This switch in firing mode is associated with a depolarization of the membrane potential of approximately 8–12 mV in thalamocortical neurons²⁵ and presumably also in the GABAergic neurons of the PGN.^{7,61} The depolarization inactivates the low-threshold Ca^{2+} current in both thalamic reticular and thalamocortical neurons, abolishes rhythmic burst firing and subsequently abolishes synchronized network oscillations such as spindle waves.

Previous investigations of cat and guinea-pig LGNd thalamocortical neurons^{46,48} and of GABAergic neurons of the thalamic reticular nucleus^{3,45} have revealed that activation of the

muscarinic, H_1 -histaminergic and α_1 -adrenoceptors in the LGNd or activation of 5-HT₂ (and possibly 5-HT_{1c}) and α_1 -adrenoceptors in the GABAergic neurons of the PGN results in a slow depolarization that is associated with a reduction in the relatively linear potassium current, I_{KL} , through a pertussis toxin-insensitive G-protein. Although the precise second messenger system mediating the slow depolarizing effects resulting from the activation of these receptors has yet to be identified, it is interesting to note that all of these receptors are known to be coupled to phosphatidylinositol turnover.⁵³

In addition to the reduction of I_{KL} , the activation of 5-HT, β -adrenergic, H_2 -histaminergic receptors on thalamocortical neurons has been demonstrated to enhance the hyperpolarization-activated cation current I_h through a shift in its voltage dependence.^{43,46,54} This enhancement of I_h can result in a reduction in the ability of thalamocortical neurons to generate rhythmic oscillations.⁴³ Recently, we have demonstrated that the enhancement of I_h in thalamocortical laminae of the LGNd can result in the abolition of spindle wave oscillations.³⁶ Presumably, this results from a reduction in the amplitude of GABAergic IPSPs arriving in thalamocortical cells, and therefore a reduction in their ability to generate rebound low-threshold Ca^{2+} spikes, which are critical to the generation of spindle waves. In addition, spindle waves are also abolished by the reduction of I_{KL} in perigeniculate GABAergic cells by NE or 5-HT.³⁶ Here, we demonstrate further that depolarization of thalamocortical neurons either through nicotinic, muscarinic or glutamate metabotropic receptors, or depolarization of perigeniculate neurons through CCK or glutamate metabotropic receptors, may also result in the abolition of spindle wave generation (see Fig. 7). This abolition of spindle wave generation results from the suppression of burst firing in either the perigeniculate or thalamocortical neurons. Dual intracellular recordings in perigeniculate and monosynaptically coupled thalamocortical cells have demonstrated that the activation of bursts of action potentials is critical to the generation of synaptic potentials that are large enough to activate low-threshold Ca^{2+} spikes in the postsynaptic

Fig. 7. Summary diagram of the receptor subtypes that are involved in the transition from slow wave sleep to waking and the associated block of spindle oscillations. (A) Glutamate, NE, 5-HT or CCK depolarize PGN neurons, thereby resulting in the abolition of spindle oscillations. The activation of nicotinic receptors also depolarizes PGN neurons, although the activation of muscarinic receptors hyperpolarizes them. Previous results have shown that activation of glutamate metabotropic, α_1 -noradrenergic, 5-HT₂-serotonergic and CCK receptors in thalamic reticular/PGN cells results in tonic depolarization, in part through the reduction of a resting "leak" potassium current, I_{KL} , and subsequent activation of a persistent Na^+ current, I_{Nap} , resulting in single spike activity. (B) ACh, glutamate, NE, 5-HT or histamine (HA) depolarize thalamocortical relay neurons, also resulting in the abolition of spindle oscillations. Previous results have shown that activation of glutamate metabotropic, α_1 -noradrenergic, H_1 -histaminergic and muscarinic receptors in thalamocortical cells results in tonic depolarization, in part through the reduction of a resting "leak" potassium current, I_{KL} . In addition, the activation of β -noradrenergic, serotonergic and H_2 -histaminergic receptors can result in the abolition of spindle waves through the enhancement of the hyperpolarization-activated cation current, I_h . These results suggest that modulation of I_{KL} is important in both thalamocortical and GABAergic PGN neurons, while modulation of I_h is important for the control of activity in thalamocortical neurons, but do not yet support a role for I_h in PGN neurons.

neuron⁶ (Kim U., Bal T. and McCormick D. A., unpublished observations). Through the reduction of I_{KL} , modulatory neurotransmitters may depolarize thalamocortical and perigeniculate neurons, resulting in the inactivation of the low-threshold Ca^{2+} currents in these cells, and therefore resulting in an abolition of spindle wave generation. Previous investigations have demonstrated that the activation of presynaptic receptors can alter the probability of transmitter release in the thalamus.^{66a} Although it is possible that such presynaptic actions contributed to the suppression of spindle waves demonstrated here, we have demonstrated previously that simple depolarization of thalamic neurons is sufficient to remove these cells from the active generation of these network oscillations.^{4,5,36}

In addition to the abolition of spindle waves, depolarization of thalamocortical neurons can result in an increase in responsiveness of these cells to activation of prethalamic inputs and an increase in the "linearity" with which these inputs are communicated to the cerebral cortex.^{9,16,23,44} Presumably, the overall depolarization of thalamic reticular cells in the transition from slow wave sleep to waking allows these cells to also participate in the accurate operation of sensory and motor processes. Our present results, along with those of previous investigations, demonstrate that the transition from slow wave sleep to waking may be controlled by a number of different ascending, descending and local neurotransmitter systems. We suggest that the firing mode of the thalamus, and the sensitivity with which the

thalamus transmits information, is of fundamental importance to the operation of the forebrain. So much so that multiple systems have access to this variable through their direct actions on thalamic neurons. The convergence of multiple neurotransmitter systems on to thalamic neurons ensures that these cells will be in or near the tonic firing mode under a variety of conditions associated with waking and attentiveness or rapid eye movement sleep. Since many of these postsynaptic responses exhibit a "ceiling effect", they occlude one another.^{3,45} Following maximal reduction of I_{KL} , for example, the addition of another neurotransmitter that reduces I_{KL} may not further depolarize thalamic neurons. Perhaps this occlusion ensures that the convergence of modulatory neurotransmitter systems on to thalamocortical neurons does not drive these cells to discharge at high rates in the lack of cortical or prethalamic inputs. It also seems reasonable to suggest that the multiple neurotransmitter systems converging on the thalamus modulate thalamocortical functions in subtle ways that are important for the accurate performance of sensory and motor processes, although these subtleties remain to be explored.

Acknowledgements—We thank Drs Uhnoh Kim and Thierry Bal for helpful discussions. This work was supported by grants from the National Institutes of Health, Life and Health Insurance Medical Research Fund, and the Klingenstein Fund. Additional information is available at <http://info.med.yale.edu/neurobiol/mccormick/mccormick.html>.

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(Accepted 2 September 1996)