High-Pass Filtering of Corticothalamic Activity by Neuromodulators Released in the Thalamus During Arousal: In Vitro and In Vivo

MANUEL A. CASTRO-ALAMANCOS AND MARIA E. CALCAGNOTTO
Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec H3A 2B4, Canada

Received 25 July 2000; accepted in final form 8 December 2000

Castro-Alamancos, Manuel A. and Maria E. Calcagnotto. High-pass filtering of corticothalamic activity by neuromodulators released in the thalamus during arousal: in vitro and in vivo. *J Neurophysiol* 85: 1489–1497, 2001. The thalamus is the principal relay station of sensory information to the neocortex. In return, the neocortex sends a massive feedback projection back to the thalamus. The thalamus also receives neuromodulatory inputs from the brain stem reticular formation, which is vigorously activated during arousal. We investigated the effects of two neuromodulators, acetylcholine and norepinephrine, on corticothalamic responses in vitro and in vivo. Results from rodent slices in vitro showed that acetylcholine and norepinephrine depress the efficacy of corticothalamic synapses while enhancing their frequency-dependent facilitation. This produces a stronger depression of low-frequency responses than of high-frequency responses. The effects of acetylcholine and norepinephrine were mimicked by muscarinic and α_2-adrenergic receptor agonists and blocked by muscarinic and α-adrenergic antagonists, respectively. Stimulation of the brain stem reticular formation in vivo also strongly depressed corticothalamic responses. The suppression was very strong for low-frequency responses, which do not produce synaptic facilitation, but absent for high-frequency corticothalamic responses. As in vitro, application of muscarinic and α-adrenergic antagonists into the thalamus in vivo abolished the suppression of corticothalamic responses induced by stimulating the reticular formation. In conclusion, cholinergic and noradrenergic activation during arousal high-pass filters corticothalamic activity. Thus, during arousal only high-frequency inputs from the neocortex are allowed to reach the thalamus. Neuromodulators acting on corticothalamic synapses gate the flow of cortical activity to the thalamus as dictated by behavioral state.

INTRODUCTION

The thalamus receives a massive input from the neocortex via corticothalamic fibers. The number of corticothalamic fibers is one order of magnitude larger than the number of thalamocortical axons, and cortical afferents to the thalamus largely outnumber the sensory input from peripheral receptors (Guillery 1969; Sherman and Guillery 1996; Steriade et al. 1997). In the somatosensory system the main type of corticothalamic fiber originates in layer VI and projects to the ventrobasal thalamus (ventral posterior medial and lateral nuclei), leaving a fiber collateral in the reticular nucleus (nRt) (Zhang and Deschenes 1997). Corticothalamic pathways have been proposed to play a role in modifying the size, strength, and selectivity of thalamocortical receptive fields and/or to establish cortico-cortical communication via the thalamus (Contreras et al. 1996; Diamond 1995; Ergenzinger et al. 1998; Guillery 1995; Krupa et al. 1999; Sillito et al. 1994; Weinberger 1995; Yuan et al. 1985). Short periods of repetitive stimulation of corticothalamic fibers at frequencies above 2 Hz produces strong synaptic facilitation or short-term potentiation in vitro (Castro-Alamancos and Calcagnotto 1999; McCormick and von Krosigk 1992; Scharfman et al. 1990; Turner and Salt 1998; von Krosigk et al. 1999) and in vivo (Deschenes and Hu 1990; Frigyesi 1972; Lindstrom and Wrobel 1990; Mishima 1992; Steriade 1999; Steriade and Timofeev 1997; Steriade and Wyzinski 1972; Tsumoto et al. 1978). A longer period of repetitive stimulation at 10 Hz induces long-term potentiation, while stimulation at 1 Hz induces long-term depression (Castro-Alamancos and Calcagnotto 1999). Thus corticothalamic synapses possess mechanisms to modify the effectiveness with which the neocortex can influence the thalamus. In addition to activity-dependent changes, corticothalamic synapses may be subject to neuromodulator-dependent changes (Gil et al. 1997; Isaacson et al. 1993; Miller 1998; Scanziani et al. 1997; Thompson et al. 1993; Wu and Saggau 1997). Several neuromodulatory systems from the brain stem and basal forebrain innervate the thalamus. In fact, neuromodulatory synapses from the brain stem and corticothalamic synapses may provide the two main synaptic inputs to the thalamus (Erisir et al. 1997). Cholinergic and noradrenergic fibers project densely to the ventrobasal thalamus and nRt (Asanuma 1997; Steriade et al. 1997). Neurons from these neuromodulatory systems discharge vigorously during behavioral activation (Aston-Jones et al. 1991; Buzsaki et al. 1988), producing effects on the firing properties of thalamic neurons that have been extensively described (McCormick 1992; Steriade et al. 1997). In contrast, their effects on synaptic transmission in the thalamus are less understood.

METHODS

In vitro methods

Thalamocortical slices were prepared from adult (≥7 wk) BALB/C mice as previously described (Agmon and Connors 1991; Castro-Alamancos and Calcagnotto 1999). Slices were cut in ice-cold buffer

Address for reprint requests: M. Castro-Alamancos, Montreal Neurological Institute, 3801 University St., Rm. WB210, Montreal, Quebec H3A 2B4, Canada (E-mail: mcastro@bic.mni.mcgill.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

using a vibratome and kept in a holding chamber for a least 1 h. Experiments were performed in an interface chamber at 32°C. The slices were perfused constantly (1–1.5 ml/min) with artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 3 KCl, 1.25 NaH_2Po_4, 26 $NaHCO_3$, 1.3 $MgSO_4$ $7H_2O$, 10 dextrose, and 2.5 $CaCl_2$ $2H_2O$. The ACSF was bubbled with 95% O_2-5% CO_2. Synaptic responses were evoked using a concentric stimulating electrode placed in the thalamic radiation unless otherwise indicated. The stimulus consisted of a 200-μs pulse of <50 μA. Field recordings from the ventrobasal thalamus were made using a low-impedance pipette ($\sim$0.5 MΩ) filled with ACSF containing 400 μM bicuculline methobromide (BMI) unless otherwise indicated. Intracellular recordings were performed using sharp electrodes (60–80 MΩ) filled with Cs^+-acetate (1 M) and QX-314 (50 mM) to suppress K^+ and Na^+ currents and postsynaptic $GABA_B$ responses. In some experiments, the $GABA_B$ receptor antagonist CGP35348 (100 μM) was also included in the bath. $GABA_A$ receptors were blocked using bath application (10–20 μM) or local application of BMI from the extracellular recording electrode or bath application of picrotoxin (10–20 μM). The test stimulus was delivered at 0.05 Hz and was either single or a pair with a 50-ms interstimulus interval to evaluate paired-pulse facilitation or four stimuli delivered at different frequencies to perform a spectrum analysis. Input resistance was measured by applying a 50- to 100-ms negative current pulse (0.3 nA). Group data are expressed as means $\pm$ SD. For single experiments, which represent typical examples, every response at 0.05 Hz is displayed. To ensure that activity in cortical circuits did not feed back to the thalamus, we severed all connections between thalamus and neocortex with a cut just below the cortical white matter (Castro-Alamancos and Calcagnotto 1999). Drugs were tested using bath application for 5–10 min unless otherwise indicated. They were prepared fresh and protected from light and from oxidation (40 μM ascorbic acid in the ACSF) as required.

In vivo methods

Adult Sprague-Dawley rats (300 g) were anesthetized with urethan (1.5 g/kg ip) and placed in a stereoaxic frame. All skin incisions and frame contacts with the skin were injected with lidocaine (2%). A unilateral craniotomy extended over a large area of the parietal cortex. Small incisions were made in the dura as necessary, and the cortical surface was covered with ACSF. Body temperature was automatically maintained constant with a heating pad. The level of anesthesia was monitored with field recordings and limb-withdrawal reflexes and kept constant at about stage III/3 using supplemental doses of urethan (Friedberg et al. 1999). Electrodes were inserted with stereotaxic procedures (all coordinates are given in mm, in reference to bregma and the dura) (Paxinos and Watson 1992). Coordinates for the ventrobasal recording electrode were anterior-posterior = −3.5, lateral = 3, and depth = 5–6. Coordinates for the thalamic radiation stimulating electrode were anterior-posterior = −2, lateral = 4, and depth = 3–4. Coordinates for the brain stem reticular formation stimulating electrode were anterior-posterior = −9, lateral = 0.7, and depth = 5–6. This stimulating electrode was placed close to the laterodorsal tegmentum and locus coeruleus to activate both cholinergic and noradrenergic fibers innervating the thalamus. Stimulation of the thalamic radiation consisted of four 200-μs pulses of <200 μA delivered at 0.1, 0.5, 1, 2, 5, 10, 20, or 40 Hz. Stimulation of the brain stem reticular formation consisted of 200-μs pulses of <300 μA delivered at 100 Hz for 1 s. A microdialysis probe was placed in the thalamus 0.5–1 mm medial from the thalamic recording electrode as previously described (Castro-Alamancos 1999). This allowed infusing drugs into the thalamus during recordings. 6-Cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX) was applied at 200 μM in the ACSF. The muscarinic antagonist scopolamine and the α-adrenergic antagonist phentolamine were applied at 500 μM in the ACSF. The Animal Care Committee of McGill University approved protocols for all experiments.

RESULTS

Field and intracellular potentials were recorded from neurons of the ventrobasal thalamus in brain slices of adult mice. Orthodromic stimuli applied to the thalamic radiation evoked a negative potential in field recordings from populations of neurons and an excitatory postsynaptic potential (EPSP) in intracellular recordings from individual neurons of the ventrobasal thalamus (Fig. 1). Some field potential recordings also display a fiber volley immediately before the synaptic negativity (Fig. 1, arrows). The fiber volley follows high-frequency stimulation and is abolished by tetrodotoxin, but not by glutamate receptor antagonists (Castro-Alamancos and Calcagnotto 1999). The field and intracellular EPSPs reflect a monosynaptic excitatory connection between corticothalamic fibers and neurons in the ventrobasal thalamus (Castro-Alamancos and Calcagnotto 1999). We explored the effects of acetylcholine and norepinephrine on corticothalamic EPSPs. To reduce the postsynaptic effects of these neuromodulators, Na^+ and K^+ currents were suppressed with the intracellular recording solution, and, to assure that the effects were not mediated by inhibition, we blocked $GABA_A$ and postsynaptic $GABA_B$ receptors. Thus while most postsynaptic actions of the neuromodulators will be blocked in the intracellular EPSPs, the extracellular field EPSPs will represent a combination of presynaptic and postsynaptic effects. The suppression of Na^+ and K^+ currents was indicated by the following observations. First, the membrane potential of neurons recorded using Cs^+ and QX-314 (-51 ± 6 mV, mean $\pm$ SD) was significantly more depolarized than that of neurons recorded using K^+-acetate (-73 ± 2 mV). Further depolarization from these values was impeded by application of negative current. Second, the input resistance of neurons recorded using Cs^+ and QX-314 (100–150 MΩ) is greater than in thalamic neurons recorded using K^+-acetate (40–60 MΩ). Third, action potentials were completely abolished, and strong positive current pulses evoked only large depolarizing plateau potentials. Finally, application of the neuromodulators was performed after extensive diffusion of the intracellular solution by waiting long periods after impalement (more than 2 h for the neurons shown in Fig. 1). Under these conditions, application of either acetylcholine (1–10 mM) or norepinephrine (10–100 μM) caused corticothalamic EPSPs to depress as reflected in the extracellular and intracellular evoked response ($n = 3$–5; Fig. 1). Corticothalamic EPSPs returned to their baseline amplitude after wash out of the drugs. The depression of corticothalamic EPSPs was not accompanied by a change in the field potential fiber volley, nor was it followed by a change in input resistance or baseline membrane potential (Fig. 1). To further eliminate the contribution of inhibition via the nRt, we produced a cut that excised this nucleus from the ventrobasal thalamus in several experiments. This assured that the norepinephrine was not depolarizing nRt cell bodies (McCormick 1992) to increase GABA release in the ventrobasal thalamus and depress corticothalamic EPSPs via presynaptic $GABA_B$ receptors. In this case corticothalamic stimulation was delivered at the nRt-ventrobasal thalamus border. To further block $GABA_B$ receptors, in some experiments we also applied CGP35348 (100 μM) in the bath. These manipulations did not

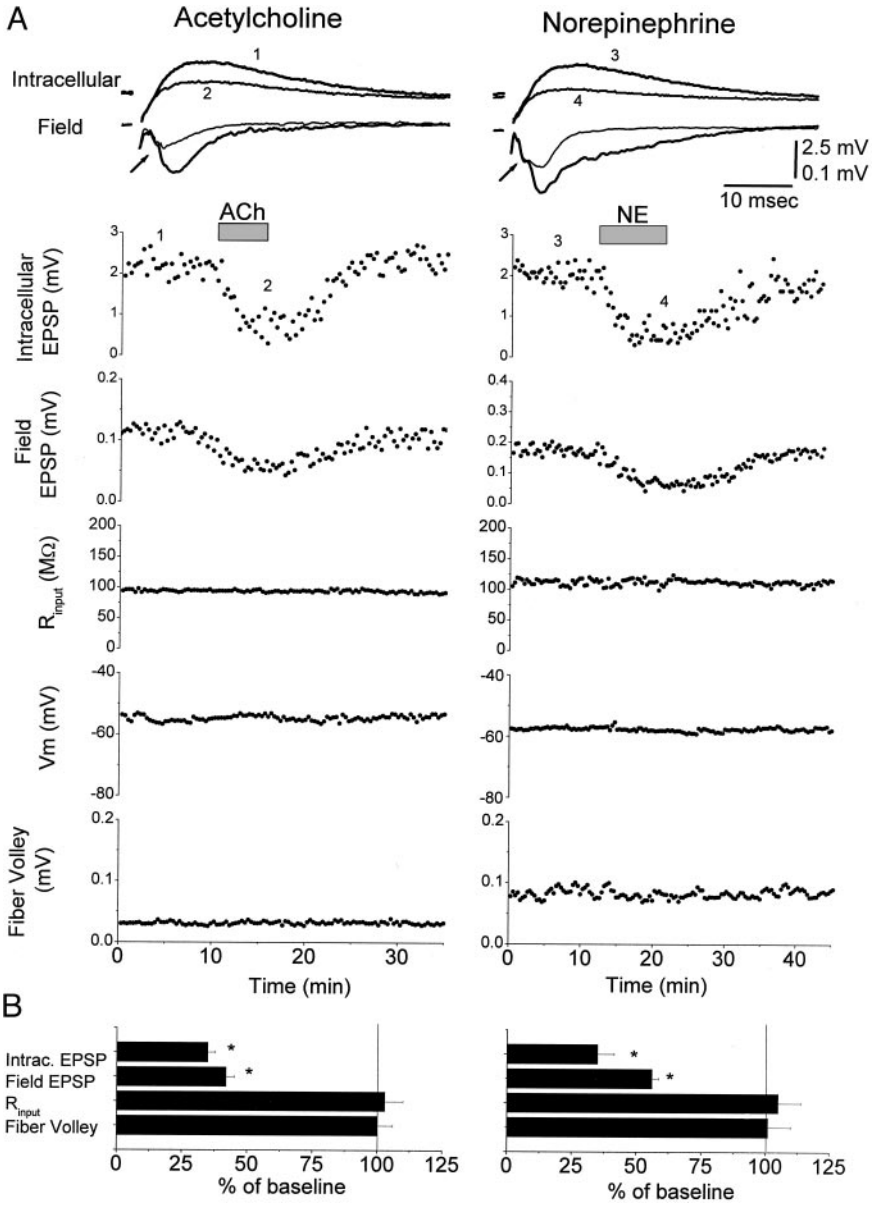

FIG. 1. Acetylcholine and norepinephrine depress corticothalamic excitatory postsynaptic potentials (EPSPs) in vitro. The effects of acetylcholine (*left*) and of norepinephrine (*right*) on the amplitude of the intracellular EPSP, the amplitude of the field EPSP, the input resistance and membrane potential of the neurons, and on the fiber volley (arrow) are subsequently displayed. The traces represent intracellular and field EPSPs recorded in the ventrobasal thalamus in response to stimulation of the thalamic radiation before and during application of acetylcholine and norepinephrine. Application of acetylcholine (ACh, 10 mM) and norepinephrine (NE, 100 μM) reversibly depressed corticothalamic EPSPs, with no significant effects on the neuron's input resistance, membrane potential, or the fiber volley. The *bottom graphs* represent the average effects of these drugs on 3–5 experiments per group as compared with baseline responses (* $P < 0.01$, *t*-test). Intracellular recordings were performed with QX-314 and Cs^+ in the pipette. Bicuculline methobromide (BMI) was bath applied.

interfere with the depression exerted by acetylcholine or norepinephrine ($n = 3$ per drug).

To test via which receptors acetylcholine (muscarinic or nicotinic) and norepinephrine (α- or β-adrenergic) depress corticothalamic EPSPs, we used receptor agonists to mimic the effects of these neuromodulators. We also used receptor antagonists to block the effects of the neuromodulators. Application of a cholinergic receptor agonist (carbachol; 5 μM, $n = 7$) or a muscarinic receptor agonist (muscarine; 10 μM, $n = 5$) depressed corticothalamic field EPSPs (Fig. 2). Nicotine (10 μM, $n = 5$) or dimethylphenylpiperazinium (10 μM, $n = 5$), which activate nicotinic receptors, had no significant effects. Application of a muscarinic receptor antagonist (scopolamine; 10 μM, $n = 5$) abolished the depression of corticothalamic field EPSPs induced by acetylcholine (10 mM). This indicates that acetylcholine depresses corticothalamic synapses by activating muscarinic receptors. Application of an α_2-adrenergic receptor agonist (clonidine, 10–40 μM, $n = 5$) also depressed corticothalamic field EPSPs (Fig. 2). In contrast, an α_1-adrenergic receptor agonist (phenylephrine; 5–50 μM, $n = 5$) and a β-adrenergic receptor agonist (isoproterenol; 10–50 μM, $n = 6$) were ineffective. Application of an α-adrenergic receptor antagonist (phentolamine; 100 μM, $n = 5$) blocked the depression of corticothalamic field EPSPs exerted by norepinephrine. This indicates that norepinephrine depresses corticothalamic synapses by activating α_2-adrenergic receptors.

Although acetylcholine and norepinephrine depress corticothalamic EPSPs, it is possible that their characteristic frequency-dependent facilitation increases. This could happen if corticothalamic depression is due to a decrease in neurotransmitter release probability. Synapses with a low release probability display stronger facilitation than synapses with a high release probability (Debanne et al. 1996; Dobrunz and Stevens 1997; Fisher et al. 1997; Manabe et al. 1993; Zucker 1989). Indeed, we found that bath application of acetylcholine or norepinephrine enhanced facilitation (Fig. 3). This was observed in the field ($n = 5$–7 per drug) and intracellular EPSPs ($n = 3$–5 per drug), was not followed by a significant change in input resis-

tance (Fig. 3*A*), and was reversible (Fig. 3*B*). The enhancement in facilitation induced by acetylcholine was mimicked by carbachol, but not by nicotine, and was blocked by the muscarinic receptor antagonist, scopolamine (Fig. 3*C*). Also, the enhancement in facilitation induced by norepinephrine was mimicked by clonidine, but not by phenylephrine or isoproterenol, and was blocked by the α-adrenergic antagonist, phentolamine.

Previous work in vivo has shown that thalamic responses can change during distinct behavioral states or after stimulation of the reticular formation (Pare et al. 1990; Steriade et al. 1969, 1986; Timofeev et al. 1996). Those studies found that ascending cerebellothalamic and mamillothalamic pathways in vivo are enhanced during arousal. The present results in vitro suggested that during arousal, when acetylcholine and norepinephrine are released into the thalamus, the descending corticothalamic pathway depresses. The next experiments explored this possibility. To induce arousal we used stimulation of the brain stem reticular formation in vivo. As previously described (Moruzzi and Magoun 1949; Steriade and McCarley 1990), this stimulation produces a strong activating effect in the thalamus consisting of the abolition of slow-wave activity and an increased depolarization that results in high-frequency unit firing (Fig. 4*A*). These effects last for at least 10 s, which was defined as our testing period (between 1 and 10 s after the reticular formation stimulation). Stimulation of the thalamic radiation in anesthetized rats produced a corticothalamic re-

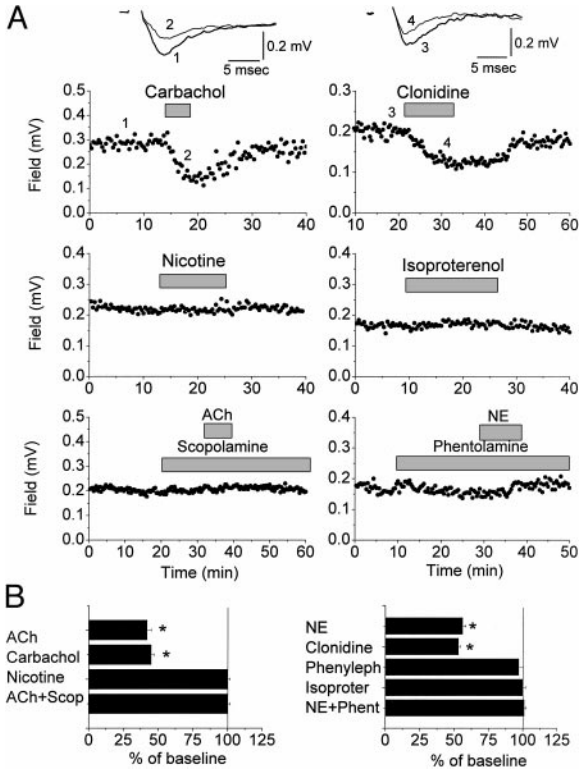

FIG. 2. Activation of muscarinic and α_2-adrenergic receptors depress corticothalamic EPSPs in vitro. The effects of cholinergic (*left*) and of noradrenergic (*right*) receptor agonists and antagonists on field EPSPs are displayed subsequently. *A*: the traces correspond to field responses recorded before and during application of the drug in the experiment displayed below. The cholinergic agonist (carbachol, 5 μM) depressed corticothalamic responses. Nicotine (10 μM) did not significantly affect corticothalamic responses. Application of a muscarinic antagonist (scopolamine, 10 μM) blocked the effects of acetylcholine (ACh, 10 mM) on corticothalamic responses. The α_2-adrenergic agonist (clonidine, 40 μM) depressed corticothalamic responses. The β-adrenergic agonist (isoproterenol, 50 μM) did not significantly affect corticothalamic responses. Application of an α-adrenergic antagonist (phentolamine, 100 μM) blocked the effects of norepinephrine (NE, 100 μM) on corticothalamic responses. *B*: average effects of the drugs on 5–7 experiments per group as compared with baseline responses (* $P < 0.01$, *t*-test). The *left panel* displays the effects of acetylcholine (10 mM), carbachol (5 μM), nicotine (10 μM), and acetylcholine in the presence of scopolamine (ACh + Scop; 10 mM + 10 μM). The *right panel* displays the effects of norepinephrine (NE, 100 μM), clonidine (40 μM), phenylephrine (50 μM), isoproterenol (50 μM), and norepinephrine in the presence of phentolamine (NE + Phent; 100 μM + 100 μM). BMI was in the recording pipette.

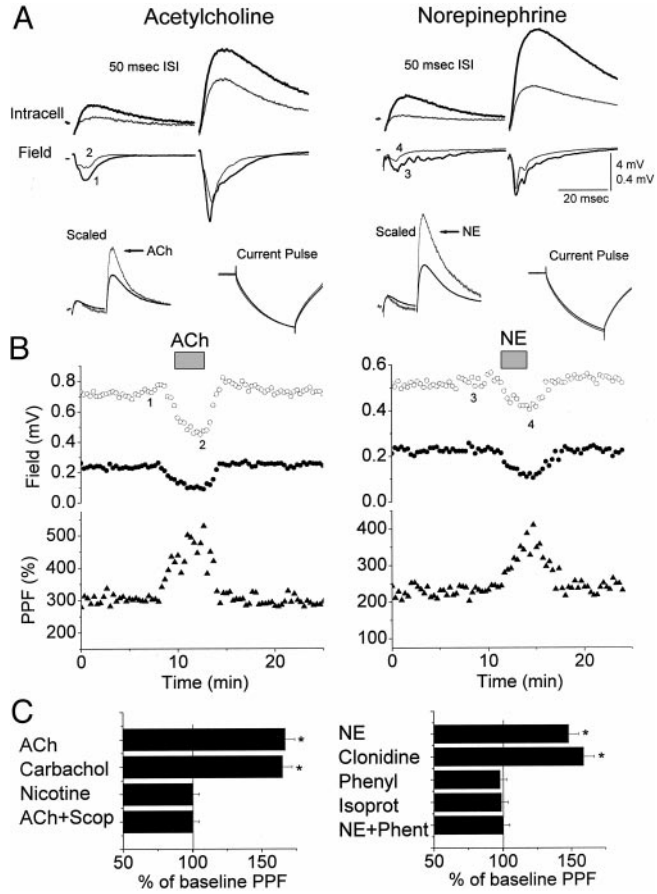

FIG. 3. Acetylcholine (*left*) and norepinephrine (*right*) enhance corticothalamic facilitation. *A*: the *top traces* represent the typical facilitation displayed by intracellular and field EPSPs in response to a pair of stimuli at a 50-ms interstimulus interval (ISI). Overlaid are responses before and during the application of acetylcholine (10 mM) and norepinephrine (100 μM). The *insets* below show the result of scaling the amplitude of the 1st (depressed) response during the drug to the amplitude of the response before the drug. This reveals an enhancement of facilitation. Also shown are the neuron's responses to a DC current pulse (−0.3 nA) delivered before and during drug application. *B*: the experiments corresponding to the extracellular traces shown in *A* are illustrated. The *top graph* shows the amplitude of the response to the 1st stimulus (●) and to the 2nd stimulus (○) delivered with a 50-ms ISI. The graph below displays (▲) the percentage of change in amplitude of the 2nd response with respect to the 1st response (PPF). Notice that during application of acetylcholine (ACh, 10 mM) and norepinephrine (NE, 100 μM), the evoked responses depressed but facilitation increased. *C*: the graphs represent the average effects of the drugs on the baseline PPF (5–7 experiments per group; * $P < 0.01$, *t*-test). The *left panel* displays the effects of acetylcholine (10 mM), carbachol (5 μM), nicotine (10 μM), and acetylcholine in the presence of scopolamine (ACh + Scop; 10 mM + 10 μM). The *right panel* displays the effects of norepinephrine (NE, 100 μM), clonidine (40 μM), phenylephrine (50 μM), isoproterenol (50 μM), and norepinephrine in the presence of phentolamine (NE + Phent; 100 μM + 100 μM). Intracellular recordings were performed with QX-314 and Cs^+ in the pipette. BMI was bath applied.

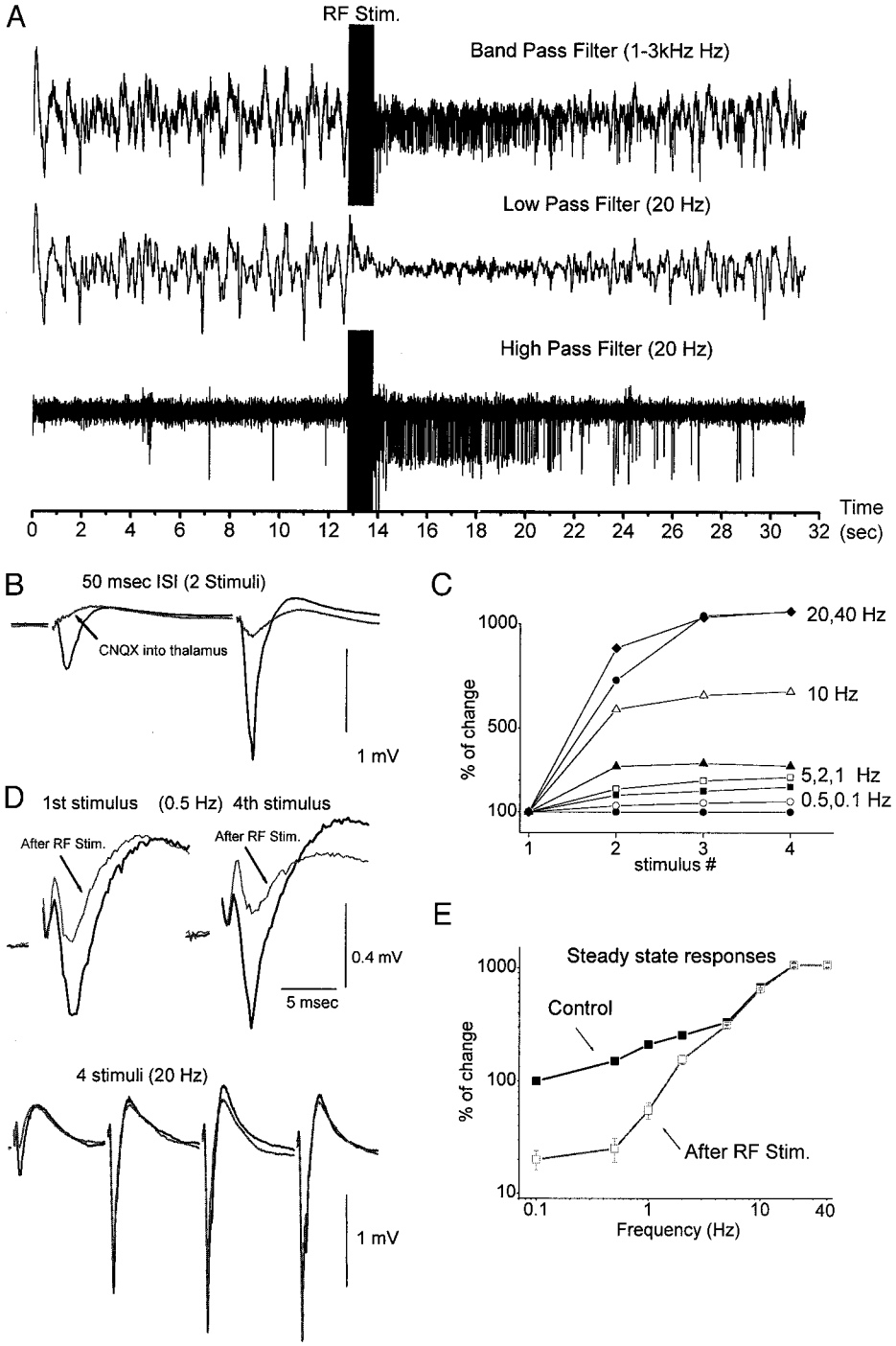

FIG. 4. Stimulation of the reticular formation in vivo suppresses low-frequency but not high-frequency corticothalamic responses. *A*: typical example of the effects of stimulating the brain stem reticular formation (RF, 100 Hz/1 s) on thalamic activity. The 3 traces correspond to the same recording with different filter settings. The low-pass filter displays slow oscillations, while the high-pass filter displays multi-unit activity. RF stimulation abolished slow oscillations and increased high-frequency neuronal discharges. *B*: stimulation of the thalamic radiation in vivo induces a corticothalamic response that is similar to those recorded in vitro. It facilitates when stimulated at a 50-ms interstimulus interval (ISI). The response is abolished by application of a non–*N*-methyl-D-aspartate (non-NMDA) glutamate receptor antagonist (CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione) into the thalamus. *C*: the amplitude of the corticothalamic responses displayed in response to 4 stimuli delivered at 0.1, 0.5, 1, 2, 5, 10, 20, and 40 Hz are displayed as a percentage of change with respect to the 1st response. *D*: the traces represent typical examples of the effects of RF stimulation on corticothalamic responses evoked at 0.5 Hz (above; only the 1st and 4th responses are shown) and at 20 Hz (below). The 20-Hz train triggers facilitation, but not the 0.5-Hz train. Overlaid are the responses before and after RF stimulation. Notice the depression of the responses at 0.5 Hz, but only of the 1st response in the 20-Hz train. *E*: as shown in *C*, facilitation reaches a steady state by the 3rd response. We used the 3rd and 4th responses of the trains to perform a spectrum analysis on the effects of RF stimulation. The average amplitude of the 3rd and 4th responses for each frequency is displayed as the percentage of change with respect to the control response (before RF) at 0.1 Hz. Notice that the steady-state low-frequency responses are abolished while the high-frequency responses are unaffected by RF stimulation. The data correspond to the average of 4 experiments.

sponse in the ventrobasal thalamus very similar to the one recorded in vitro (compare Figs. 3*A* and 4*B*), with its characteristic strong facilitation (Fig. 4*C*). As previously observed in vitro (Castro-Alamancos and Calcagnotto 1999), the corticothalamic response was abolished in vivo by application of a glutamate receptor antagonist into the thalamus (Fig. 4*B*). This shows that antidromic activation does not contribute to the synaptic field response we measure because this response is completely abolished by CNQX. Also, antidromic excitation in the ventrobasal thalamus cannot produce recurrent EPSPs because thalamocortical neurons are not synaptically connected to each other. We first tested the effects of arousal on the corticothalamic pathway by comparing responses before and after reticular formation stimulation ($n = 7$). Corticothalamic responses were strongly depressed during the testing period (Fig. 4*D*). This depression usually lasted between 20 and 60 s. Thus as predicted from the effects of acetylcholine and norepinephrine in vitro, stimulation of the reticular formation resulted in a strong depression of the corticothalamic pathway.

Does this mean that during arousal the cortex cannot communicate with the thalamus? One possibility is that corticothalamic responses only depress at low frequencies, but not at high frequencies that engage facilitation. This was suggested by the observation, in vitro, that acetylcholine and norepineph-

rine enhance facilitation. Thus we compared the effects of reticular formation stimulation on low-frequency and on high-frequency corticothalamic activity (Fig. 4*D*). The results showed that while the corticothalamic responses to low-frequency activity were strongly depressed by stimulating the reticular formation, the responses to high-frequency activity were not affected. A spectrum analysis revealed that corticothalamic responses below 5 Hz were strongly suppressed during arousal, but higher frequency responses were unaffected. (Fig. 4*E*). The steady-state responses at a frequency below 5 Hz were significantly depressed by stimulation of the reticular formation ($n = 4$; *t*-test, $P < 0.01$). These results indicate that during arousal corticothalamic activity is high-pass filtered at 5 Hz. Moreover, consistent with the in vitro results, application of both muscarinic and α-adrenergic antagonists into the thalamus abolished the depression of corticothalamic responses induced by stimulating the reticular formation ($n = 3$; Fig. 4*F*). This experiment also allowed dissociating the synaptic effects from the activating effects of reticular formation stimulation. Figure 5 shows the effect of stimulating the reticular formation before (ACSF) and during the application of both muscarinic and α-adrenergic antagonists into the thalamus (scopolamine and phentolamine). The reticular formation stimulation was effective under both conditions in producing thalamic activation (Fig. 5*A*). This was expected because it is known that many different neuromodulators produce activating effects in the thalamus and that acetylcholine acting via nicotinic receptors and norepinephrine via β-adrenergic receptors (which were not blocked) also depolarize thalamic neurons (McCormick 1992; Steriade et al. 1997). Although application of scopolamine and phentolamine did not eliminate the thalamic activation produced by stimulating the reticular formation, it did abolish the depression of corticothalamic responses (Fig. 5*B*). This indicates that the depressing effect on corticothalamic synapses induced by stimulating the reticular formation is independent from the changes in cellular excitability of thalamic activation.

We also tested the effects of reticular formation activation on thalamic responses evoked by stimulating the medial lemniscus (Castro-Alamancos, unpublished observations) and found that these responses were either enhanced or not affected ($n = 4$; not shown) as previously described (Steriade and Demetrescu 1960; for review Singer 1977; Steriade and McCarley 1990; Steriade et al. 1997). This suggests that the depressing effects of stimulating the reticular formation are restricted to corticothalamic synapses. Therefore the effects in vivo on corticothalamic responses induced by stimulating the reticular formation are likely synaptic and not a general consequence of a change in excitability of thalamic neurons.

Finally, we tested whether activation of cholinergic or α_2-adrenergic receptors in slices using receptor agonists was sufficient to high-pass filter corticothalamic responses in vitro. To mimic our conditions in vivo, we recorded corticothalamic field responses in the absence of GABA receptor antagonists.

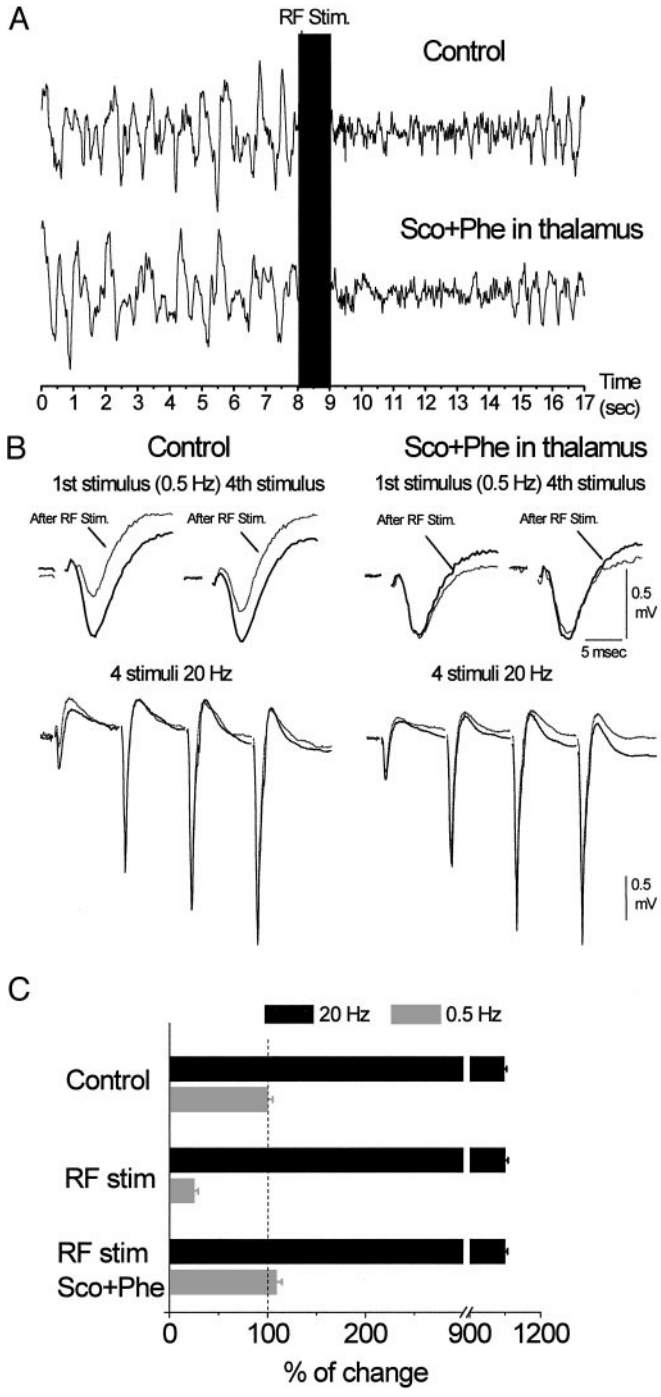

FIG. 5. Blocking muscarinic and α-adrenergic receptors in the thalamus dissociates between the synaptic and cellular excitability effects of stimulating the reticular formation. *A*: typical examples showing similar effects of stimulating the brain stem reticular formation (RF, 100 Hz/1 s) on thalamic activity during application of artificial cerebrospinal fluid (ACSF; control) or during application of scopolamine and phenolamine (500 μM; Sco + Phe) into the thalamus. Traces are low-pass filtered. *B*: differential effects of stimulating the brain stem reticular formation on corticothalamic responses during application of ACSF (control; *left*) or during application of scopolamine and phentolamine into the thalamus (Sco + Phe; *right*). The traces represent typical examples of the effects of RF stimulation on corticothalamic responses evoked at 0.5 Hz (above; only the 1st and 4th responses are shown) and at 20 Hz (below). The 20-Hz train triggers facilitation, but not the 0.5-Hz train. Overlaid are the responses before and after RF stimulation in the presence (*right*) and absence (*left*) of scopolamine and phentolamine in the thalamus. Notice that in the presence of Sco + Phe, stimulation of the reticular formation does not depress corticothalamic responses at 0.5 Hz or the 1st response of the train at 20 Hz. All data in this figure are from the same experiment. *C*: group data ($n = 3$) shown are the mean amplitudes of the steady-state responses for a low- (0.5) and a high-frequency (20 Hz) stimulus displayed as the percentage of the control (before RF) response at 0.1 Hz. RF stimulation depresses selectively the low-frequency response, and infusing scopolamine and phentolamine (500 μM) in the thalamus blocks this effect.

Under these conditions corticothalamic field responses recorded in slices are similar to those recorded in vivo (compare Figs. 4*D* and 6*A*), with the characteristic strong facilitation (Fig. 6*B*). The neuromodulator agonists were applied locally (at the recording site) using a low-impedance pipette containing the drug (at a dose 10 times higher than in the bath) dissolved in ACSF as previously described (Castro-Alamancos and Calcagnotto 1999). Application of either clonidine (400 μM, $n = 3$) or carbachol (50 μM, $n = 3$) resulted in a strong depression of the corticothalamic responses (Fig. 6*A*). Low-frequency responses were always affected more than high-frequency responses (Fig. 6*A*). This effect resembles the result obtained by stimulating the reticular formation in vivo. As in vivo, we performed a spectrum analysis, which revealed that low-frequency corticothalamic responses were strongly suppressed but high-frequency responses were unaffected by local application of clonidine or carbachol (Fig. 6*C*). The steady-state responses at a frequency below 10 Hz were significantly depressed by carbachol or clonidine ($n = 3$; *t*-test, $P < 0.01$). It is unlikely that we would be able to completely simulate in vitro the exact pattern and dosage of receptor activation that corresponds to stimulating the brain stem reticular formation in vivo. Several differences were noted between the in vivo and in vitro experiments. First, the $GABA_A$ receptor–dependent positivity that follows the negative field potential EPSP, which is only observed in the absence of BMI, showed strong frequency-dependent depression in vitro but not in vivo. Second, the frequency-dependent facilitation displayed in vivo and in vitro is very similar but not identical. Facilitation in vivo tends to be stronger and begins at higher frequencies than in vitro. This is likely due to differences in the experimental conditions (e.g., ACSF). Surely, these factors contribute to the differences between the frequency curves of the steady-state responses in vivo and in vitro. Taken together, the results in vitro and the antagonist experiments in vivo indicate that activation of muscarinic or α_2-adrenergic receptors at corticothalamic synapses during arousal result in the selective suppression of low-frequency corticothalamic activity.

DISCUSSION

The present experiments, performed in vitro and in vivo, reveal that acetylcholine and norepinephrine depress corticothalamic synapses via muscarinic and α_2-adrenergic receptors. However, since corticothalamic depression is followed by an enhancement of facilitation, only low-frequency corticothalamic activity is suppressed during arousal, while high-frequency activity, which triggers facilitation, flows unaffected.

The results indicate that the release of acetylcholine or norepinephrine in the thalamus depresses corticothalamic synapses by activating muscarinic and α_2-adrenergic receptors, respectively. First, acetylcholine and norepinephrine depress corticothalamic EPSPs measured intra- and extracellularly. This depression occurs both in the presence or absence of GABA-mediated inhibition. Second, cholinergic and α_2-adrenergic receptor agonists, but not nicotinic or β-adrenergic agonists, depress corticothalamic EPSPs. Third, a muscarinic antagonist and a α-adrenergic antagonist block the depressing effects of acetylcholine and norepinephrine on corticothalamic EPSPs, respectively. Fourth, stimulation of the reticular formation, which releases several neuromodulators into the thalamus, depresses corticothalamic field EPSPs, and this depres-

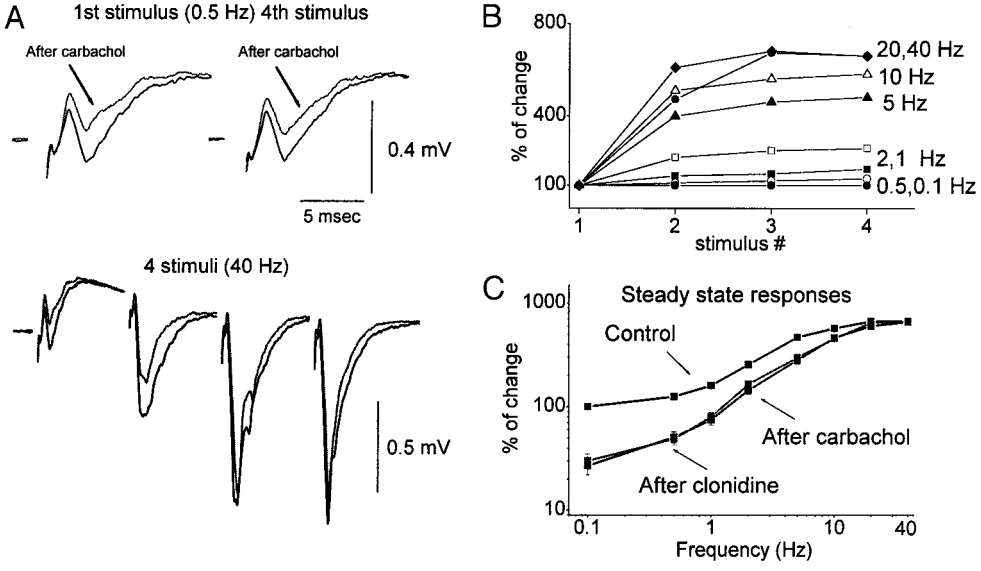

FIG. 6. Application of muscarinic or α_2-adrenergic receptor agonists in vitro suppresses low-frequency but not high-frequency corticothalamic responses. *A*: the traces represent typical examples of the effects of carbachol on corticothalamic responses evoked at 0.5 Hz (above; only the 1st and 4th responses are shown) and at 40 Hz (below). The 40-Hz train triggers facilitation, but not the 0.5-Hz train. Overlaid are the responses before and during carbachol. Notice the depression of the responses at 0.5 Hz, but only of the 1st and 2nd responses in the 40-Hz train. *B*: the amplitude of the corticothalamic responses displayed in response to 4 stimuli delivered at 0.1, 0.5, 1, 2, 5, 10, 20, and 40 Hz are displayed as a percentage of change with respect to the 1st response. *C*: as shown in *B*, facilitation reaches a steady state by the 3rd response. We used the 3rd and 4th responses of the trains to perform a spectrum analysis on the effects of carbachol and clonidine. The average amplitude of the 3rd and 4th responses for each frequency is displayed as the percentage of change with respect to the control response (before the drug) at 0.1 Hz. Notice that the steady-state low-frequency responses are abolished while the high-frequency responses are unaffected by clonidine and carbachol. The data correspond to the average of 3 experiments.

sion is abolished by the application of muscarinic and α-adrenergic antagonists into the thalamus.

Previous work has demonstrated that corticothalamic synapses are depressed by glutamate via type III metabotropic glutamate receptors (Turner and Salt 1999). The present study ads both muscarinic and α_2-adrenergic receptors to the list of neuromodulators that regulate corticothalamic communication. By which mechanisms do muscarinic and α_2-adrenergic receptors depress corticothalamic EPSPs? A differential presynaptic modulation of low-frequency versus high-frequency synaptic activity by muscarine and by GABA has been previously shown in the ventral striatum (Pennartz and Lopes da Silva 1994) and in the auditory system (Brenowitz et al. 1998), respectively. The present results also imply that corticothalamic depression induced by acetylcholine and norepinephrine is due to a presynaptic mechanism involving a decrease in the probability of neurotransmitter release. This is suggested by the finding that both neuromodulators produced an enhancement in paired-pulse facilitation, which is consistent with a decrease in neurotransmitter release probability (Manabe et al. 1993; Zucker 1989). One mechanism that cannot account for the effects of the neuromodulators on facilitation is a change in the frequency-dependent depression of disynaptic inhibitory postsynaptic potentials (von Krosigk et al. 1999) because, in our experiments, inhibition was blocked. Moreover, a postsynaptic effect of these neuromodulators consisting in a change in cellular excitability is unlikely to explain the synaptic depression because we significantly suppressed the postsynaptic actions of these neuromodulators during intracellular recordings by blocking Na^+ and K^+ conductances, and this did not affect the synaptic depression. However, a postsynaptic action of the neuromodulators at corticothalamic synapses cannot be ruled out. Of particular interest was the dissociation in vivo between the effects of muscarinic and α-adrenergic antagonists on corticothalamic responses and on thalamic activation. These antagonists specifically abolished the depression of low-frequency corticothalamic responses induced by stimulating the reticular formation, but not the thalamic activation. This suggests that the well-known postsynaptic depolarizing effects produced by the release of neuromodulators on thalamic neurons are not contributing to the corticothalamic depression. The dissociation between thalamic activation and the depression of corticothalamic responses was also supported by the observation that the corticothalamic depression always outlasted the thalamic activation. Finally, the observation that only corticothalamic, but not medial lemniscus, responses are depressed by stimulating the reticular formation demonstrates that the effect is input specific. This suggests that the depressing effect of the neuromodulators is occurring at corticothalamic synapses and is not a general consequence of a change in excitability. Thus the present results indicate that the depression occurs at corticothalamic synapses. Further work should clarify whether the locus of depression is presynaptic or postsynaptic.

What is the functional significance of a decrease in corticothalamic efficacy that is accompanied by an increase in facilitation? The thalamocortical system undergoes dramatic functional changes between sleep and arousal (Steriade et al. 1997). For example, during slow-wave sleep the neocortex and thalamus are engaged in low-frequency oscillations, while during arousal they engage in high-frequency *gamma* oscillations (Steriade et al. 1993). Gamma oscillations at 20–40 Hz coincide with the frequencies that are effective in triggering short-term synaptic facilitation in corticothalamic synapses. Interestingly, stimulating the brain stem reticular formation enhances both gamma oscillations (Steriade and Amzica 1996; Steriade et al. 1991) and synaptic facilitation. Thus an important role for this corticothalamic high-pass filter during arousal is to permit the flow of gamma oscillations between neocortex and thalamus, while impeding low-frequency oscillations. It would serve as a gate that allows the flow of low-frequency and gamma activity during sleep, while allowing only gamma activity during arousal.

We thank Dr. Steriade for helpful comments.

This research was supported by the Medical Research Council of Canada, the Natural Sciences and Engineering Council of Canada, Fonds de la Reserche en Sante du Quebec, the Canadian Foundation for Innovation, and the Savoy Foundation.

REFERENCES

AGMON A AND CONNORS BW. Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience* 41: 365–379, 1991.

ASANUMA C. Distribution of neuromodulatory inputs in the reticular and dorsal thalamic nuclei. In: *Thalamus*, edited by Steriade M, Jones EG, and McCormick DA. New York: Elsevier, 1997, vol. 2, p. 93–154.

ASTON-JONES G, CHIANG C, AND ALEXINSKY T. Discharge of noradrenergic locus coeruleus neurons in behaving rats and monkeys suggests a role in vigilance. *Prog Brain Res* 88: 501–520, 1991.

BRENOWITZ S, DAVID J, AND TRUSSELL L. Enhancement of synaptic efficacy by presynaptic GABA(B) receptors. *Neuron* 20: 135–141, 1998.

BUZSAKI G, BICKFORD RG, PONOMAREFF G, THAL LJ, MANDEL R, AND GAGE FH. Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. *J Neurosci* 8: 4007–4026, 1988.

CASTRO-ALAMANCOS MA. Neocortical synchronized oscillations induced by thalamic disinhibition in vivo. *J Neurosci* 19: RC27, 1–7, 1999.

CASTRO-ALAMANCOS MA AND CALCAGNOTTO ME. Presynaptic long-term potentiation in corticothalamic synapses. *J Neurosci* 19: 9090–9097, 1999.

CONTRERAS D, DESTEXHE A, SEJNOWSKI TJ, AND STERIADE M. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science* 274: 771–774, 1996.

DEBANNE D, GUERINEAU NC, GAHWILER BH, AND THOMPSON SM. Paired-pulse facilitation and depression at unitary synapses in rat hippocampus: quantal fluctuation affects subsequent release. *J Physiol (Lond)* 491: 163–176, 1996.

DESCHENES M AND HU B. Electrophysiology and pharmacology of the corticothalamic input to lateral thalamic nuclei: an intracellular study in the cat. *Eur J Neurosci* 2: 140–152, 1990.

DIAMOND ME. Somatosensory thalamus of the rat. In: *Cerebral Cortex*, edited by Jones EG and Diamond IT. New York: Plenum, 1995, vol. 11, p. 189–219.

DOBRUNZ LE AND STEVENS CF. Heterogeneity of release probability, facilitation, and depletion at central synapses. *Neuron* 18: 995–1008, 1997.

ERGENZINGER ER, GLASIER MM, HAHM JO, AND PONS TP. Cortically induced thalamic plasticity in the primate somatosensory system. *Nature Neurosci* 1: 226–229, 1998.

ERISIR A, VAN HORN SC, AND SHERMAN SM. Relative numbers of cortical and brainstem inputs to the lateral geniculate nucleus. *Proc Natl Acad Sci USA* 94: 1517–1520, 1997.

FISHER SA, FISCHER TM, AND CAREW TJ. Multiple overlapping processes underlying short-term synaptic enhancement. *Trends Neurosci* 20: 170–177, 1997.

FRIEDBERG MH, LEE SM, AND EBNER FF. Modulation of receptive field properties of thalamic somatosensory neurons by the depth of anesthesia. *J Neurophysiol* 81: 2243–2252, 1999.

FRIGYESI TL. Intracellular recordings from neurons in dorsolateral thalamic reticular nucleus during capsular, basal ganglia and midline thalamic stimulation. *Brain Res* 48: 157–172, 1972.

GIL Z, CONNORS BW, AND AMITAI Y. Differential regulation of neocortical synapses by neuromodulators and activity. *Neuron* 19: 679–686, 1997.

GUILLERY RW. A quantitative study of synaptic interconnections in the dorsal lateral geniculate nucleus of the cat. *Z Zellforsch* 96: 39–48, 1969.

GUILLERY RW. Anatomical evidence concerning the role of the thalamus in corticocortical communication. A brief review. *J Anat* 187: 583–592, 1995.

ISAACSON JS, SOLIS JM, AND NICOLL RA. Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron* 10: 165–175, 1993.

KRUPA DJ, GHAZANFAR AA, AND NICOLELIS MA. Immediate thalamic sensory plasticity depends on corticothalamic feedback. *Proc Natl Acad Sci USA* 96: 8200–8205, 1999.

LINDSTROM S AND WROBEL A. Frequency dependent corticofugal excitation of principal cells in the cat's dorsal lateral geniculate nucleus. *Exp Brain Res* 79: 313–318, 1990.

MANABE T, WYLLIE DJ, PERKEL DJ, AND NICOLL RA. Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J Neurophysiol* 70: 1451–1459, 1993.

MCCORMICK DA. Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. *Prog Neurobiol* 39: 337–388, 1992.

MCCORMICK DA AND VON KROSIGK M. Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. *Proc Natl Acad Sci USA* 89: 2774–2778, 1992.

MILLER RJ. Presynaptic receptors. *Annu Rev Pharmacol Toxicol* 38: 201–227, 1998.

MISHIMA K. Facilitatory and inhibitory processes in the thalamic ventrobasal nucleus of the rat. *Jpn J Physiol* 42: 193–210, 1992.

MORUZZI G AND MAGOUN HW. Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* 1: 455–473, 1949.

PARE D, STERIADE M, DESCHENES M, AND BOUHASSIRA D. Prolonged enhancement of anterior thalamic synaptic responsiveness by stimulation of a brain-stem cholinergic group. *J Neurosci* 10: 20–33, 1990.

PAXINOS AG AND WATSON C. *The Rat Brain in Stereotaxic Coordinates.* New York: Academic, 1992.

PENNARTZ CM AND LOPES DA SILVA FH. Muscarinic modulation of synaptic transmission in slices of the ventral striatum is dependent on the frequency of afferent stimulation. *Brain Res* 645: 231–239, 1994.

SCANZIANI M, SALIN PA, VOGT KE, MALENKA RC, AND NICOLL RA. Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors. *Nature* 385: 630–634, 1997.

SCHARFMAN HE, LU SM, GUIDO W, ADAMS PR, AND SHERMAN SM. *N*-methyl-D-aspartate receptors contribute to excitatory postsynaptic potentials of cat lateral geniculate neurons recorded in thalamic slices. *Proc Natl Acad Sci USA* 7: 4548–4552, 1990.

SHERMAN SM AND GUILLERY RW. Functional organization of thalamocortical relays. *J Neurophysiol* 76: 1367–1395, 1996.

SILLITO AM, JONES HE, GERSTEIN GL, AND WEST DC. Feature-linked synchronization of thalamic relay cell firing induced by feedback from the visual cortex. *Nature* 369: 479–482, 1994.

SINGER W. Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiol Rev* 57: 386–420, 1977.

STERIADE M. Coherent oscillations and short-term plasticity in corticothalamic networks. *Trends Neurosci* 22: 337–345, 1999.

STERIADE M AND AMZICA F. Intracortical and corticothalamic coherency of fast spontaneous oscillations. *Proc Natl Acad Sci USA* 93: 2533–2538, 1996.

STERIADE M AND DEMETRESCU M. Unspecific systems of inhibition and facilitation of potentials evoked by intermittent light. *J Neurophysiol* 23: 602–617, 1960.

STERIADE M, DOMICH L, AND OAKSON G. Reticularis thalami neurons revisited: activity changes during shifts in states of vigilance. *J Neurosci* 6: 68–81, 1986.

STERIADE M, DOSSI RC, PARE D, AND OAKSON G. Fast oscillations (20–40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *Proc Natl Acad Sci USA* 88: 4396–4400, 1991.

STERIADE M, IOSIF G, AND APOSTOL V. Responsiveness of thalamic and cortical motor relays during arousal and various stages of sleep. *J Neurophysiol* 32: 251–265, 1969.

STERIADE M, JONES EG, AND MCCORMICK DA. *Thalamus.* New York: Elsevier, 1997, vol. 1.

STERIADE M AND MCCARLEY RW. *Brainstem Control of Wakefulness and Sleep.* New York: Plenum, 1990.

STERIADE M, MCCORMICK DA, AND SEJNOWSKI TJ. Thalamocortical oscillations in the sleeping and aroused brain. *Science* 262: 679–685, 1993.

STERIADE M AND TIMOFEEV I. Short-term plasticity during intrathalamic augmenting responses in decorticated cats. *J Neurosci* 17: 3778–3795, 1997.

STERIADE M AND WYZINSKI P. Cortically elicited activities in thalamic reticularis neurons. *Brain Res* 42: 514–520, 1972.

THOMPSON SM, CAPOGNA M, AND SCANZIANI M. Presynaptic inhibition in the hippocampus. *Trends Neurosci* 16: 222–227, 1993.

TIMOFEEV I, CONTRERAS D, AND STERIADE M. Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. *J Physiol (Lond)* 494: 265–278, 1996.

TSUMOTO T, CREUTZFELDT OD, AND LEGENDY CR. Functional organization of the corticofugal system from visual cortex to lateral geniculate nucleus in the cat (with an appendix on geniculo-cortical mono-synaptic connections). *Exp Brain Res* 32: 345–364, 1978.

TURNER JP AND SALT TE. Characterization of sensory and corticothalamic excitatory inputs to rat thalamocortical neurons in vitro. *J Physiol (Lond)* 510: 829–843, 1998.

TURNER JP AND SALT TE. Group III metabotropic glutamate receptors control corticothalamic synaptic transmission in the rat thalamus in vitro. *J Physiol (Lond)* 519: 481–491, 1999.

VON KROSIGK M, MONCKTON JE, REINER PB, AND MCCORMICK DA. Dynamic properties of corticothalamic excitatory postsynaptic potentials and thalamic reticular inhibitory postsynaptic potentials in thalamocortical neurons of the guinea-pig dorsal lateral geniculate nucleus. *Neuroscience* 91: 7–20, 1999.

WEINBERGER NM. Dynamic regulation of receptive fields and maps in the adult sensory cortex. *Annu Rev Neurosci* 18: 129–158, 1995.

WU LG AND SAGGAU P. Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* 20: 204–212, 1997.

YUAN B, MORROW TJ, AND CASEY KL. Responsiveness of ventrobasal thalamic neurons after suppression of S1 cortex in the anesthetized rat. *J Neurosci* 5: 2971–2978, 1985.

ZHANG ZW AND DESCHENES M. Intracortical axonal projections of lamina VI cells of the primary somatosensory cortex in the rat: a single-cell labeling study. *J Neurosci* 17: 6365–6379, 1997.

ZUCKER RS. Short-term synaptic plasticity. *Annu Rev Neurosci* 12: 13–31, 1989.