**Physiology and Pharmacology of Corticothalamic Stimulation-Evoked Responses in Rat Somatosensory Thalamic Neurons In Vitro**

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Kao, Chang-Qing and Douglas A. Coulter. Physiology and pharmacology of corticothalamic stimulation-evoked responses in rat somatosensory thalamic neurons in vitro. *J. Neurophysiol.* 77: 2661–2676, 1997. Whole cell current- and voltage-clamp recording techniques were employed in a rat thalamocortical slice preparation to characterize corticothalamic stimulation-evoked responses in thalamic neurons. Three types of corticothalamic stimulation-evoked responses were observed in thalamic neurons. Of thalamic neurons, 57% responded to corticothalamic stimulation with purely excitatory synaptic responses, whereas 27% had inhibitory synaptic responses and 16% had mixed excitatory/inhibitory responses. This suggested corticothalamic activation of multiple distinct synaptic circuits, presumably involving both nucleus reticularis thalami (NRT) and thalamus, because the rat ventrobasal complex is virtually devoid of GABAergic interneurons. Corticothalamic-stimulation-evoked excitatory postsynaptic currents (EPSCs) were predominantly slow rising currents that showed nonlinear voltage dependence, characteristics of an N-methyl-D-aspartate (NMDA)-receptor-mediated synaptic current. These slow rising EPSCs were blocked by the NMDA antagonist 2-amino-5-phosphonovaleric acid (APV). A minority of corticothalamic EPSCs had faster kinetics, and were blocked by 6-cyano-7 nitroquinoxaline-2,3-dione (CNQX). Corticothalamic stimulation of varying frequency optimally activated burst responses in thalamic neurons at low frequencies (3–6 Hz). The optimal 3–6-Hz response was reduced by ethosuximide, by APV, and by detaching the neocortex from the thalamocortical slice, suggesting that T current, NMDA receptors, and neocortical properties all contributed to generation of this 3–6-Hz frequency preference. In contrast to corticothalamic EPSCs, medial-thalamic-stimulation-evoked responses consisted of fast CNQX-sensitive EPSCs that were predominantly voltage insensitive, with no 3–6-Hz frequency preference. In thalamic neurons in which corticothalamic stimulation evoked predominantly inhibitory synaptic responses, this inhibitory postsynaptic potential (IPSP) had early and late phases, often followed by a rebound burst. The early IPSP reversed at –95 mV and was bicuculline sensitive, whereas the late IPSP reversed at –113 mV and was blocked by the γ-aminobutyric acid-B (GABA_B) antagonist 3-N[1-(S)-(3,4-di-chlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphonic acid (CGP-55845A). In thalamic neurons in which corticothalamic stimulation evoked a mixed excitatory postsynaptic potential (EPSP)/IPSP response, repetitive corticothalamic stimulation rapidly reduced IPSPs and enhanced EPSPs at higher frequencies. This resulted in burst firing being triggered in these mixed response neurons at frequencies >6 Hz. Corticothalamic feedback onto thalamic relay neurons activated diverse responses due to differing relative activation of NRT and ‘‘feedforward’’ inhibitory responses. These multiple in vitro corticothalamic responses differ from responses encountered in other in vitro thalamic preparations lacking a synaptically connected neocortex, but are similar to results evident in thalamic neurons in response to cortical stimulation in vivo. In addition, the thalamocortical 3–6-Hz frequency preference was conserved, suggesting that many factors critical for this emergent property of the thalamocortical system are maintained in vitro.

**INTRODUCTION**

Corticothalamic feedback onto thalamic relay neurons plays a significant role in modulating normal peripheral sensory inputs, and is also intimately involved in generation of thalamocortical rhythms. Thalamocortical rhythms underlie both physiological and pathophysiological events including sleep spindles (Andersen et al. 1967; Steriade and Llinas 1988) and generalized absence seizures (Niedermeyer 1990; Steriade et al. 1993; Williams 1953). Corticothalamic feedback may be particularly critical in generation of the spike-wave discharges of generalized absence seizures, which require functional connectivity between cortex and thalamus for expression, unlike spindle discharges, which can occur in decorticate animals (Vergnes and Marek 1992; for review see Gloor and Fariello 1988; Steriade et al. 1993).

Rodent somatosensory cortical neurons of layer VI and lower layer V innervate topographically appropriate regions of the ventrobasal (VB) complex of thalamus (Chmielowska et al. 1989). Activation of these corticothalamic feedback projections provides both direct excitatory input to VB neurons and indirect inhibitory input via collaterals to the nucleus reticularis thalami (NRT), a nucleus composed of exclusively of GABAergic neurons (Houser et al. 1980), which in turn innervates the thalamus (Bourassa and Deschenes 1995; Steriade et al. 1984; Yen et al. 1985). Activation of primary somatosensory corticothalamic neurons facilitated somatosensory responses in VB neurons (Yuan et al. 1985, 1986), and in vivo field potential and single-unit recording experiments in rats, corticothalamic axons could be activated from contralateral somatosensory cortex, and triggered excitation followed by inhibition of VB neurons (Mishima 1992). In addition, repetitive corticothalamic activation enhanced corticothalamic responses (Mishima 1992). This augmenting response was blocked by local microinjection of kynurenic acid into VB, to antagonize corticothalamic glutamatergic transmission (Mishima and Ohtaka 1992). Physiological, pharmacological, and immunocytochemical experimental findings suggest that the corticothalamic projection may be glutamatergic (Baughman and Gil-
Stimulation of corticothalamic fibers resulted in monosynaptic excitation of lateral geniculate nucleus thalamic relay neurons mediated via activation of ionotropic glutamate receptors (Deschenes and Hu 1990; Scharfman et al. 1990). Therefore it is likely that corticothalamic synaptic transmission in VB neurons is also glutamatergic. In mouse, immunogold staining demonstrated that VB neurons, large "specific" somatosensory terminals, and small corticothalamic terminals exhibit only glutamate-like immunoreactivity. A third terminal type (presumably NRT synaptic profiles) stained only for γ-aminobutyric acid (GABA) (Hamori et al. 1990).

There are few published reports physiologically and pharmacologically characterizing the properties of corticothalamic synaptic potentials in somatosensory thalamus with the use of high-resolution intracellular recording techniques. In the present study, we employ a rat thalamocortical slice that maintains reciprocal connections between somatosensory thalamus, cortex, and NRT to examine the nature of corticothalamic synaptic responses in VB neurons, with a specific focus on assessing the potential contribution of these synapses to development of oscillatory thalamocortical processes. This slice is adapted from the mouse thalamocortical slice preparation described by Agmon and Connors (1991). A preliminary report of these findings has been published in abstract form (Kao and Coulter 1995).

METHODS

Male or female Sprague-Dawley rats (16–28 days postnatal) were anesthetized and decapitated, and brains were dissected rapidly. Thalamocortical slices 450 μm thick were cut with the use of a Vibratome at the slice angle described by Agmon and Connors (1991). Slices were placed in an interface recording chamber and studies in which thalamic responses to cortical stimulation of varying intensity were examined, a 2-s stimulus train was employed. Only neurons with stable membrane potentials negative to −55 mV were included in the present study. Data were digitized with the use of a pulse-code modulation device (Neurodata, Greenvale, NY) and stored on videotape. Data were played back, digitized, and analyzed off-line with the use of pClamp software (Axon Instruments, Foster City, CA).

Cortical-stimulation-evoked excitatory postsynaptic currents (EPSCs)/excitatory postsynaptic potentials (EPSPs) in VB neurons were characterized electrophysiologically and pharmacologically. To characterize voltage-dependent block of N-methyl-D-aspartate (NMDA)-receptor-mediated components of EPSCs by Mg2+ (Mayer et al. 1984; Nowak et al. 1984), membrane potential was systematically varied between −80 and 30 mV. In voltage-clamp experiments in which synaptic responses were examined, a computer-generated step potential protocol was designed to inactivate the thalamic low-threshold calcium current (LTCC) before each synaptic stimulation, to avoid contamination of the synaptic event by activation of calcium conductances and to facilitate isolation of synapticly activated currents. Bicuculline methiodide (10–20 μM) was added to the extracellular solution in most voltage-clamp recordings to block GABA A -receptor-mediated responses to facilitate the isolation of EPSCs. Under these conditions, stimulation intensity was carefully controlled and minimized to ensure that epileptic events were not activated in the disinhibited cortex by the stimulation. In some experiments, extracellular recordings were used to monitor for such events. The NMDA receptor dependence of components of the EPSCs was confirmed by application of the specific NMDA antagonist 2-amino-5-phosphonovaleric acid (APV, 50 μM). When α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-receptor-mediated components of EPSCs were to be studied, membrane potential was held at −80 mV to block NMDA components, or the sensitivity of the EPSC to the specific non-NMDA excitatory amino acid receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; HBC complex, 20 μM) was examined.

RESULTS

Cortical-stimulation-evoked responses were studied in 61 VB neurons in current-clamp mode and in 35 neurons in voltage-clamp mode. All neurons had stable membrane potentials. The average resting membrane potential of these neurons was −66 ± 3.5 (SD) mV and the average input resistance was 329 ± 21 MΩ. All recorded neurons responded with a burst of action potentials to depolarizing current injection and most triggered rebound bursting in response to a hyperpolarizing current pulse.

Three populations of cortical-stimulation-evoked responses were observed in VB neurons. In 57% (35 of 61) of neurons, corticothalamic stimulation evoked responses that were exclusively excitatory, consisting of EPSPs and EPSP-evoked bursts of action potentials (Fig. 1). In 26% (16 of 61) of neurons, corticothalamic stimulation evoked responses that were inhibitory, consisting of inhibitory post-synaptic potentials (IPSPs), which occasionally triggered rebound low-threshold calcium spikes (see below). In the remainder of neurons (16%, or 10 of 61), corticothalamic stimulation elicited mixed EPSP/IPSP responses (see below). For the purposes of clarity in presentation, results in these three populations of corticothalamic responses in VB neurons (excitatory, inhibitory, and mixed) will be presented separately.
Corticothalamic-stimulation-evoked excitatory responses in VB relay neurons

Stimulation of deep layers of cortex (layers V and VI) with single pulses usually elicited short-latency EPSPs in the “excitatory” subpopulation of VB neurons (57% of recorded cells) at fairly low thresholds (0.5–1 V). These EPSPs often triggered regenerative conductances in these neurons, presumably mediated by the large LTCCs present in thalamic neurons (e.g., Fig. 1A). In neurons in which regenerative conductances were not activated by the cortical-stimulation-evoked EPSP at lower stimulus intensities, increasing the strength of the stimulation gradually increased the amplitude of the EPSP until in virtually all cases a low-threshold calcium-dependent burst was triggered in VB neurons (Fig. 1B), either directly by the EPSP (Fig. 1A) or temporally linked to the EPSP (Fig. 1B). These later bursts could be triggered by delayed polysynaptic cortical processes activated by the stimulus (e.g., Coulter and Lee 1993). This relationship of stimulus intensity to amplitude of response is plotted in Fig. 1C. Threshold intensity stimulation evoked a small EPSP (Fig. 1B, top trace). As the intensity of stimulation was increased, larger-amplitude EPSPs were evoked, until a plateau amplitude was reached at >1.5 V, where increasing stimulation evoked delayed bursts of action potentials (Fig. 1B, bottom trace).

To characterize the nature of the synaptic current activated by corticothalamic excitatory transmission, VB neurons were recorded in voltage-clamp mode, and the EPSC was isolated with cesium-based electrode solution together with extracellular perfusion of 20 μM bicuculline. VB neurons are endowed with a large LTCC (Coulter et al. 1989a), which could be readily activated synaptically and was difficult to clamp, particularly at the 34°C recording temperatures employed in the present study. Particular attention was paid to blocking the contribution of unclamped LTCC to synaptically evoked currents, to avoid confounding analysis of these events. With the use of a computer-generated protocol, the LTCC was completely inactivated by holding the neuron’s membrane potential at −20 mV for 250 ms before stepping to various potentials and recording synaptic currents activated by corticothalamic stimulation. This was found to be
sufficient time to completely inactivate the LTCC, and only 100 ms separated termination of the inactivation protocol and stepping to various potentials to record corticothalamic EPSCs. This is insufficient time to allow the LTCC to recover from inactivation (Coulter et al. 1989a). A sufficient concentration of bicuculline was used to ensure the blockade of GABA_A conductances (20 μM) yet not trigger massive spontaneous cortical epileptic activity (which frequently occurred at concentrations >20 μM). In addition, the corticothalamic stimulus intensity was kept as low as possible, so as not to evoke cortical epileptic events. Under these recording conditions, most cortical-stimulation-evoked EPSCs were relatively slow rising, long-duration inward currents (evident in 24 of 28 VB neurons in voltage-clamp recordings). A representative example of these slow EPSCs is depicted in Fig. 2. After inactivation of the LTCC, the holding potential was stepped to various potentials (−80 to +20 mV), low-amplitude corticothalamic stimuli were delivered, and the voltage dependence of the resulting synaptic current was plotted (Fig. 2A). At about −50 mV, the evoked current reached a maximum amplitude, and either depolarizing or hyperpolarizing the holding potential from this point reduced the amplitude of the EPSC (Fig. 2A and C). This EPSC was completely blocked by bath application of the NMDA antagonist APV (50 μM). Figure 2C is a plot of the corticothalamic EPSC response amplitude at varying holding potentials in the absence (●) or presence (■) of APV. Note the strong voltage dependence and APV sensitivity of the corticothalamic evoked EPSC in this cell. These prevalent slow rising, voltage-dependent, APV-sensitive corticothalamic EPSCs are consistent with strong contributions of bicuculline was used to ensure the blockade of GABA_A conductances (20 μM) yet not trigger massive NMDA receptor activation to EPSCs in VB neurons in response to corticothalamic synaptic activity. Only 4 of 28 VB neurons responded to corticothalamic stimulation with fast rising EPSCs (Fig. 3). In these neurons, the fast EPSCs were partially blocked by bath application of the non-NMDA antagonist CNQX (20 μM) (Fig. 3).

Repetitive corticothalamic-stimulation-evoked bursts in VB neurons optimally in a frequency range of 3–6 Hz

Repetitive corticothalamic stimulation (2-s trains at 1–15 Hz) in excitatory response VB neurons maximally evoked large-amplitude bursting responses in a very restricted frequency range (Fig. 4). In these experiments, a corticothalamic stimulation input/output curve was constructed in the neuron to be tested, and the stimulus intensity was adjusted to a level of 50% of maximal (the stimulus level that triggered bursting) for repetitive activation studies. As is shown in Fig. 4A, 1-Hz corticothalamic stimulation only evoked
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tions, including sleep spindles and the spike-wave discharges of generalized absence seizures.

Corticothalamic-stimulation-evoked low-frequency bursting in VB neurons is blocked by ethosuximide and APV

To better understand factors contributing to the low-frequency optimal bursting responses in VB neurons to corticothalamic stimulation, various drugs were applied to assess potential effects on the tuning curve. Considering the time course of these responses (optimal responses at 3 Hz, or at 300-ms intervals), several processes could contribute to determining this refractory period between bursts. One possible contributor is the thalamic LTCC, which rapidly inactivates after activation and has a 250-ms time constant for recovery from inactivation (Coulter et al. 1989a). This obviously could limit the frequency of calcium current activation (and bursting) in VB neurons. Other factors could include 1) the relatively long-lasting NMDA current activated during the corticothalamic EPSP and 2) the network properties of the neocortex, which is also activated by the corticothalamic stimulation. Several experiments were carried out to test these hypotheses. Ethosuximide is a selective generalized absence anticonvulsant that specifically blocks thalamic LTCC in clinically relevant concentration ranges (250–1,000 μM) (Coulter et al. 1989b). Ethosuximide’s effects on repetitive corticothalamic stimulation responses were tested in seven VB neurons. A representative example is depicted in Fig. 5. This neuron showed a typical optimal-frequency bursting response at 3 Hz in control medium (Fig. 5A, left). After application of ethosuximide, this optimal low-frequency response was selectively dampened (in fact, completely blocked), without any change evident in higher-frequency responses (Fig. 5A, middle), and these effects of ethosuximide were readily reversible (Fig. 5A, right).

Plotting this corticothalamic tuning curve response in Fig. 5B, the selective reduction of the optimal low-frequency response pattern becomes apparent. Similar effects of ethosuximide were seen in a total of seven VB cells. These evoked in VB neurons by corticothalamic stimulation. B and C: this fast EPSC was partially blocked by application of 20 μM CNQX. D: this block was reversible (holding potential: –80 mV).

EPSPs in this neuron. When administered at 3 Hz, the same magnitude of corticothalamic stimuli evoked four large bursts of action potentials in response to six stimuli. Similarly, 6-Hz stimulation elicited bursts of action potential in response to 5 of 12 stimuli. Increasing the frequency of stimulation beyond 6 Hz failed to further enhance the burst responses. In fact, a marked decrement in these responses was evident at all frequencies >6 Hz (Fig. 1A). This relationship of stimulus frequency to percent of stimulation-evoked bursts is plotted in Fig. 4B. Optimal numbers of burst responses were triggered in response to corticothalamic stimuli in the 3- to 6-Hz frequency range. Similar corticothalamic stimulation frequency tuning curve results were seen in 13 excitatory response category VB neurons. This cortical stimulation “tuning curve” and its frequency range overlapped with the frequency of various thalamocortical oscillations, including sleep spindles and the spike-wave discharges of generalized absence seizures.

FIG. 3. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX)-sensitive corticothalamic EPSCs. A: in 4 of 28 VB neurons, a faster rising EPSC was evoked in VB neurons by corticothalamic stimulation. B and C: this fast EPSC was partially blocked by application of 20 μM CNQX. D: this block was reversible (holding potential: –80 mV).
bution of cortical responses to the optimal low-frequency thalamic response to repetitive corticothalamic stimulation, a series of thalamocortical slices was cut in which the cortex was removed by a scalpel cut. In these decorticate slices, striatal stimulation activation of corticothalamic fibers (as close to the edge of cortical cut as possible) could not evoke a similar low-frequency bursting response to that seen in cortical stimulation in intact slices \((n = 4\) VB neurons). In Fig. 7A, current-injection-evoked bursting could be elicited in the recorded neuron, but no optimal low-frequency-stimulation-evoked bursting was evident, although large-amplitude EPSPs could be triggered (Fig. 7B). Similar results were seen in four VB neurons. These results indicate that activation of intrinsic cortical properties may also facilitate optimal low-frequency response patterns in response to repetitive corticothalamic stimulation.

**Effects of local lemniscal afferent stimulation in VB neurons**

In contrast to corticothalamic-stimulation-evoked EPSCs, EPSCs evoked in response to local stimulation within VB (presumably activating lemniscal sensory afferent fibers) (Agmon and Connors 1991) evoked mainly fast rising EPSCs in VB neurons \((n = 7)\). These lemniscal EPSCs (Fig. 8A) showed little voltage dependence, with linear variations in response amplitude between −80 and 0 mV (Fig. 8, A and B). These lemniscal EPSCs were partially blocked by bath application of the non-NMDA antagonist CNQX (Fig. 8C; \(n = 7\)). Lemniscal EPSC response properties were markedly different from corticothalamic-stimulation-evoked EPSCs recorded in the same neurons (e.g., Fig. 2).

Repetitive activation of the local lemniscal inputs elic-
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**FIG. 5.** Ethosuximide selectively blocked corticothalamic-stimulation-evoked low-frequency bursts in VB relay neurons. A, left: control traces of 1-, 3-, and 10-Hz cortical-stimulation-evoked bursts in a VB neuron. A, middle: effects of 700 μM ethosuximide on the same stimulation frequencies as in control conditions. Note that the low-frequency bursts elicited by 3-Hz stimuli were completely blocked. A, right: wash of the ethosuximide effects. B: plot of corticothalamic stimulus frequency vs. percent of stimulation-evoked bursts in a VB neuron under control and ethosuximide-exposed conditions. Note the selective block of bursts in response to 3- to 6-Hz cortical stimulation.

Corticothalamic-stimulation-evoked inhibitory synaptic responses in VB relay neurons

In 42% of VB neurons, corticothalamic stimulation evoked either an inhibitory response (27% of neurons) or a mixed excitatory/inhibitory response (16% of neurons). In a representative example of these “inhibitory” response cells, single cortical stimulation evoked only an IPSP (Fig. 10a). These IPSPs were biphasic, with early and late components, and sometimes triggered rebound bursting (Fig. 10, a and e). In some cases, corticothalamic stimulation triggered two or more IPSPs, activated at ~3 Hz (Fig. 10d). In the rodent, there are virtually no inhibitory interneurons in VB (Ottersen and Storm-Mathisen 1984; Spreafico et al. 1994; reviewed in Jones 1985). Therefore, given that the cortical input is glutamatergic, this inhibitory input must come from feedforward activation of NRT by the corticothalamic axons. Sup-
Fig. 6. Effects of the NMDA antagonist APV on corticothalamic-stimulation-evoked low frequency bursts in a VB neuron. A: corticothalamic evoked bursts at different stimulation frequencies (1, 3, and 10 Hz) under control (left), APV-exposed (middle), and wash (right) conditions. Note the block of both EPSPs and stimulation-evoked bursting at all frequencies. B: plot of the corticothalamic stimulus frequency vs. percent of stimulation-evoked bursts in a VB neuron under control and APV-exposed conditions. Note the nonselective block of bursts in response to all frequencies of cortical stimulation.

Supporting this, similar large-amplitude IPSPs could be evoked in VB neurons by direct NRT stimulation (Fig. 11).

Corticothalamic-stimulation-evoked IPSPs had two components (Figs. 12a and 13), an early fast component that was selectively blocked by the GABA<sub>A</sub> antagonist bicuculline (Fig. 12c) and a later slow component that was selectively (and irreversibly) blocked by the GABA<sub>B</sub> antagonist 3-<br>\[N[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzylphoshinic acid (CGP-55845A; Fig. 13b) or 2-hydroxysaclofen (not shown). Looking at the activation curve for corticothalamic-evoked IPSPs (Fig. 13a), two main facts were evident. First, both the GABA<sub>A</sub> and GABA<sub>B</sub> components appeared to be activated simultaneously in response to all stimuli, varying from threshold to maximal responses (Fig. 13, a and b). Second, the intensity/amplitude of response curve had clear plateaus, or steps, rather than a smooth sigmoidal shape (Fig. 13b). NRT neurons are known to have large LTCCs (Huguenard and Prince 1994b) and to trigger bursts in response to activation (De Curtis et al. 1989). Activation of these regenerative bursts in NRT neurons at a certain threshold of corticothalamic stimulation may explain this stepwise activation curve. The reversal potentials of the early and late corticothalamic stimulation-evoked IPSPs differed. The early, fast GABA<sub>A</sub> component reversed at \(-98 \pm 6\) (SD) mV \((n = 5)\), whereas the later GABA<sub>B</sub> component reversed at \(116 \pm 5\) mV \((n = 5)\).
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**A** VB Neuron Recording in De-corticate Slice

**B** Striatum Stimulation

**FIG. 7.** Effects of removal of the cortex in thalamocortical slices on the tendency to elicit bursting in response to low-frequency corticothalamic stimulation. **A:** recordings from a VB neuron illustrate that this cell is capable of generating bursts in response to hyperpolarizing (left) and depolarizing (right) current injection. **B:** in this VB neuron, repetitive stimulation of corticothalamic fibers in the striatum did not exhibit selective bursting in the 3-Hz frequency range evident in intact slices.

**DISCUSSION**

In the present study, whole cell recording techniques were employed in rat thalamocortical slices to characterize responses elicited by activation of corticothalamic feedback onto VB neurons. Three types of corticothalamic-stimulation-evoked synaptic responses were evident: predominantly excitatory (57% of cells), predominantly inhibitory (27% of cells), and mixed (16% of cells). In the excitatory response population, voltage-clamp and pharmacological experiments revealed that corticothalamic-stimulation-evoked EPSCs were mainly mediated by activation of NMDA receptors (Fig. 2). Initially subthreshold corticothalamic EPSPs could elicit burst firing in thalamic neurons optimally in response to low-frequency (3 Hz) stimulation (Figs. 4–6). Key factors in determining this optimal frequency response to corticothalamic activation in VB neurons were 1) the intrinsic LTCC properties of thalamic neurons (Fig. 5) and 2) cortical circuit properties (Fig. 7), with a more minor contribution provided by NMDA receptor activation (Fig. 6). The corticothalamic-stimulation-evoked inhibitory responses were mediated by interspersed activation of NRT (Fig. 11), and consisted of biphasic IPSPs mediated by activation of both GABA_A and GABA_B receptors (Figs. 10 and 12). These corticothalamic-stimulation-evoked disynaptic IPSPs in VB neurons demonstrate the existence of an important inhibitory pathway contributing to the properties of corticothalamic feedback.

**Corticothalamic evoked excitatory responses**

The point-to-point precise anatomic specificity of the corticothalamic projection to VB neurons (Chmielowska et al. 1989; Ramon-Moliner 1986) indicates this pathway is a closely coupled feedforward-feedback network important in regulation of thalamocortical activity. In the present experiment, 57% of the corticothalamic-stimulation-evoked re-
FIG. 8. Local lemniscal stimulation evoked faster rising CNQX-sensitive EPSCs in VB neurons. A: local lemniscal stimulation evoked fast EPSCs in a VB thalamic neuron, with linear voltage dependence, in contrast to the slower EPSCs with nonlinear voltage dependence evoked by corticothalamic stimulation. B: plot of the current-voltage curve for the lemniscal EPSC shows nearly linear responses at various holding potentials. C: lemniscal EPSC was CNQX sensitive.

Responses in VB neurons were excitatory. The evoked EPSP had a relatively low threshold, and only slightly increasing the intensity of stimulation above threshold usually evoked a burst of action potentials (Fig. 1). This indicated that corticothalamic feedback powerfully modulates the behavior of VB neurons, at least in the membrane potential ranges encountered in these in vitro recordings. Voltage-clamp studies of isolated corticothalamic stimulation-evoked EPSCs supported the role of glutamate as the corticothalamic neurotransmitter, because these EPSCs were blocked by glutamatergic antagonists (Figs. 2 and 3). This is supported by many other studies (Baughman and Gilbert 1980; Deschenes and Hu 1990; Fonnum et al. 1981; Scharfman et al. 1990; von Krosigk and McCormick 1992). However, in VB neurons, the kinetics, voltage dependence, and pharmacology of the corticothalamic EPSC were consistent with its mediation primarily via activation of NMDA receptors (Fig. 2). This is in contrast to studies in rat NRT neurons, in which cortical inputs activated a conventional two-component EPSP: an early, short-lasting, APV-insensitive portion, and a late, APV-sensitive decay phase (De Curtis et al. 1989). Corticothalamic EPSCs observed in the present study were slow rising and APV sensitive in 24 of 28 VB neurons, and pains were taken to ensure that these slow kinetics were not due to contamination of the EPSC by an unclamped regenerative calcium current. The nature of this relatively slow rising current, its characteristic voltage-dependent properties, and its sensitivity to block by APV all fit with the concept that corticothalamic EPSCs are primarily mediated by activation of NMDA receptors (Mayer et al. 1984; Nowak et al. 1984). This predominant NMDA receptor activation by corticothalamic stimulation contrasted with responses in the same neurons to local stimulation medial to the recorded VB neuron, which presumably activated sensory (lemniscal) afferents to thalamus. These responses were larger and much faster than corticothalamic EPSCs, had linear voltage dependence be-
FIG. 9. Effects of lemniscal stimulation of varying frequency on VB neuron responses. A: traces illustrating the effects of local lemniscal stimulation at 1, 3, 6, 10, 12, and 15 Hz. In contrast to corticothalamic stimulation, lemniscal stimulation at gradually escalating stimulus frequencies evoked an accumulating depolarization that triggered rapid tonic action potential firing. B: plot of lemniscal stimulus frequency vs. percent of stimulation-evoked bursts in a VB neuron. Note the lack of selective enhancement of low-frequency responses.

tween −80 and 0 mV, and were more sensitive to blockade by the non-NMDA receptor antagonist CNQX (Fig. 8). In other studies, it has been demonstrated that in response to activation of sensory afferents in rat, cat, and ferret dorsal lateral geniculate nucleus, the generation of thalamic output is mainly controlled by activation of non-NMDA receptors, whereas the contribution of NMDA receptors is limited to the burst firing generated by the low-threshold calcium spike (Ramoa and McCormick 1994; Turner et al. 1994). In the present study, thalamic burst firing was driven primarily by cortical feedback activating an NMDA-receptor-activation-mediated EPSC (Figs. 2 and 6).

The voltage dependence of the slow corticothalamic-stimulation-evoked EPSC was shifted to more hyperpolarized levels in thalamic neurons (unblock occurring at −50 mV) (Fig. 2) compared with NMDA-receptor-dependent EPSCs in hippocampal neurons (unblock at −30 mV) (e.g., Hestrin et al. 1990; Keller et al. 1991). One potential mechanism that might contribute to this difference is alterations in the subunit composition of the NMDA receptors in the thalamus relative to the neocortex and hippocampus. The thalamus has relatively equivalent levels of expression of NR2A, NR2B, NR2C, and NR2D subunit mRNAs, whereas the neocortex and hippocampus lack the NR2C subunit (Monyer et al. 1992, 1994; but see Buller et al. 1994; Ishii et al. 1993). In cloning expression studies, coexpression of the NR1 and NR2A subunits resulted in expression of mediators that were less sensitive to extracellular Mg2+ and that exhibited voltage-dependent unblock at approximately −30 mV. In contrast, NR1-NR2C subunit combinations resulted in expression of receptors that were more sensitive to extracellular Mg2+ and that exhibited voltage-dependent unblock at more hyperpolarized potentials (−50 mV) (Ishii et al. 1993; Monyer et al. 1992, 1994). Assuming that higher levels of NR2C mRNA expression in the thalamus translates into higher levels of incorporation of NR2C subunits into NMDA receptors, then...
FIG. 10. Corticothalamic-stimulation-evoked inhibitory postsynaptic potentials (IPSPs) in VB neurons. a–e: traces illustrating IPSPs evoked by single cortical stimulation (5 V). The IPSP often triggered a rebound burst, and sometimes ≥1 recurrent IPSPs were triggered at ~3 Hz (membrane potential: −65 mV).

This could explain at least in part the altered voltage dependence of NMDA-receptor-mediated EPSCs in these cells (Fig. 2). A similar hyperpolarized shift in the voltage dependence of APV-sensitive EPSCs has been reported in developing neocortical neurons, and also attributed to potential heterogeneity of NMDA receptors (Burgard and Hablitz 1993), with some experimental support in the form of developmental in situ hybridization studies of NMDA receptor subunit mRNAs (e.g., Monyer et al. 1994).

In the present study, although kinetically slow NMDA-dependent EPSCs were evident in response to corticothalamic activation, no very slow, metabotropic glutamate receptor (mGlur)-dependent EPSCs or EPSPs were evident, as were seen by McCormick and von Krosigk (1992) in a study of corticothalamic tract stimulation responses in guinea pig lateral geniculate nucleus. In the present study, the lack of mGlur responses was apparent even for repetitive corticothalamic activation experiments (Figs. 4–6), in which no evidence of activation of a slow depolarization was visible even following 15-Hz stimulation. There are a number of possible explanations for these different findings. McCormick and von Krosigk (1992) usually stimulated corticothalamic tract fibers at 50 Hz to evoke slow mGlur responses, although they report that smaller-amplitude mGlur responses could be triggered by stimuli at as low a frequency as 3 Hz. In addition, McCormick and von Krosigk employed “sharp” electrodes, which would not have dialedized the neuron’s internal contents. In the present study, corticothalamic stimulus frequencies were maximally 15 Hz, so this may not have been optimal to release sufficient neurotransmitter to see the very slow, often small-amplitude metabotropic EPSPs. The neocortex was the stimulus site in the present study, which resulted in more restricted activation of smaller numbers of axons than did corticothalamic tract activation, as was conducted by McCormick and von Krosigk (1992). This could also result in reduced amounts of neurotransmitter release, which could reduce activation of mGlurs, particularly if these receptors are extrasynaptic. In addition, whole cell recording techniques were employed in the present study, which may have washed out some of
FIG. 11. IPSPs evoked by cortical, striatal, and nucleus reticularis thalami (NRT) stimulation in a VB neuron. **Left**: cortical-stimulation-evoked IPSP in a VB neuron. These IPSPs were probably due to cortical stimulation activating NRT, which in turn inhibits the thalamus because no intrinsic GABAergic interneuron are present in rodent VB. **Middle**: moving the stimulating electrode closer to the thalamus elicited larger IPSPs. **Right**: stimulating directly in NRT evoked very large IPSPs, which triggered rebound bursting in some cases (membrane potential: −59 mV).

The intracellular intermediaries necessary to evoke mGluR responses. However, GTP and ATP were included in the patch electrodes in the present study, so washout of mGluR responses is not necessarily occurring.

Corticothalamic modulation of low-frequency bursts on VB neurons

One of the major properties of corticothalamic feedback responses in VB was the selective amplification of responses during low-frequency (3 Hz) stimulation (Figs. 2–4). Similar low-frequency amplification of responses was evident in thalamic recordings during corticothalamic stimulation in vivo in cat, and repeated administration of these stimuli eventually culminated in spike-wave afterdischarges in the thalamus (Steriade et al. 1976). In vivo, phasic bursting constitutes one of the two main thalamic relay neuron firing modes, and this phasic bursting tends to occur spontaneously at low frequencies in the thalamicocortical system under certain conditions. The transition between phasic and tonic firing modes in thalamus is related to the behavioral state of the animal, in which during drowsiness, slow-wave sleep and certain epileptic seizures, phasic firing predominates, whereas tonic firing is characteristic of an awake and alert state (Steriade et al. 1990, 1993). It is reasonable to hypothesize that the low-frequency bursting activity evident in VB neurons in response to repetitive corticothalamic activation under hyperpolarized resting potentials recorded in this in vitro thalamocortical slice in the absence of sensory input may resemble the thalamic phasic firing behavior seen in vivo during drowsiness, sleep, or epileptic seizures. Because of the long time constant of recovery of activation of the LTCC in thalamic neurons (~250 ms) (Coulter et al. 1989a), one would predict that this conductance might contribute to low- but not high-frequency burst activity in thalamus. This prediction is supported by experiments in which the anticonvulsant ethosuximide blocked the corticothalamic optimal low-frequency bursting pattern in thalamic neurons (Fig. 5). This drug is a specific LTCC blocker (Coulter et al. 1989b), and is effective in control of the spike-wave discharges of absence epilepsy, which are pathological 3-Hz thalamocortical oscillations (Williams 1953; reviewed in Gloo and Fariello 1988). This LTCC, found in virtually all thalamic neurons (Deschénes et al. 1982; Jahnsen and Llinás 1984; Llinás and Jahnsen 1982), has been shown to be of primary importance in the generation of thalamocortical oscillations (reviewed in Steriade and Llinás 1988). By contrast, the NMDA antagonist APV showed only partial efficacy in blocking thalamic amplification of low-frequency corticothalamic feedback, indicating a less primary role for NMDA receptor activation in determining this frequency selectivity (Fig. 6).
The contribution of cortical mechanisms to the selective amplification of low-frequency inputs was supported by experiments in “decorticate” thalamocortical slices, in which amplification of low-frequency responses was compromised (Fig. 7). In in vivo studies, cortical pyramidal cells are hypothesized to be entrained into low-frequency oscillations, and to in turn modulate NRT and thalamic neurons and contribute to the generation of delta (1–4 Hz) slow rhythms, because corticothalamic volleys potentiate and synchronize the delta oscillation of simultaneously recorded thalamic cells (Steriade et al. 1991). The present study supports this concept, because corticothalamic-stimulation-evoked low-frequency bursts in VB neurons were significantly modulated by corticothalamic feedback onto thalamic neurons (Fig. 7). Factors within the neocortex that could conceivably be involved in selective augmentation of low-frequency thalamocortical rhythms include cortical augmenting processes (e.g., Morison and Dempsey 1942), decrement of intracortical inhibition by repeated stimulation (e.g., McCarren and Alger 1985), selective augmentation of intracortical excitatory connections by low-frequency stimulation (e.g., Thompson 1986), activation of bursting responses in deep layer neurons by repetitive stimulation (Connors and Gutnick 1990; McCormick et al. 1985), and activation of intrinsic cortical oscillatory processes (e.g., Flint and Connors 1996; Silva et al. 1991), among many possibilities.

In contrast, repetitive activation of sensory afferents to the thalamus via local stimulation evoked gradually increasing levels of depolarization and tonic action potential firing rather than bursts (Fig. 9). This corresponds more to the relay or tonic firing mode of thalamic neurons seen in vivo, and indicates that the synaptic properties of sensory and corticothalamic inputs to thalamic neurons may be quite different. This was reflected in the kinetics and pharmacology of the two responses. The lemniscal EPSC was fast, showed linear voltage dependence, and was sensitive to CNQX (Fig. 8), whereas the corticothalamic EPSC was slower, showed marked nonlinearities in voltage dependence, and was sensitive to APV (Fig. 2). These results indicate that corticothalamic and lemniscal inputs to VB neurons were different in terms of network properties and in the nature of neurotransmitter receptor subtypes activated. No polysynaptic IPSPs were evoked by local (putative lemniscal) stimulation within the thalamus. One might expect such events to occur if this stimulation could secondarily activate NRT and trigger IPSPs through trisynaptic activation (VB to NRT to VB). Given that stimulation strengths were relatively low in the present study, and extracellular Mg$^{2+}$ levels were fairly high (1.5–2 mM), this would tend to discourage this type of polysynaptic activation within the slice.

**Cortical evoked inhibitory responses**

In some VB neurons, corticothalamic stimulation evoked IPSPs with early and late components, which were mediated by activation of GABA$_A$ and GABA$_B$ receptors, respectively (Crunelli and Leresche 1991; Huguenard and Prince 1994b). The early component was bicuculline sensitive and reversed at 80 min after drug application. Note early and late components.

![Corticothalamic-stimulation-evoked IPSPs had early and late components that were sensitive to specific $\gamma$-aminobutyric acid-A (GABA$_A$) and GABA$_B$ receptor blockers, respectively.](http://jn.physiology.org/)

**FIG. 12.** Corticothalamic-stimulation-evoked IPSPs had early and late components that were sensitive to specific $\gamma$-aminobutyric acid-A (GABA$_A$) and GABA$_B$ receptor blockers, respectively. a: cortical-stimulation-evoked IPSP in control media. Note early and late components. b: late IPSP component was blocked by the GABA$_A$ receptor antagonist 3-N1-(S)-(3,4-dichlorophenyl)ethylamino-2-(S)-hydroxypropyl-P-benzylphosphonic acid (CGP-55845A; 100 nM). c: early IPSP component was blocked by the GABA$_A$ receptor antagonist bicuculline (20 $\mu$M). d: wash 80 min after drug application. Note that the bicuculline’s effects were reversible, whereas those of CGP-55845A were not (membrane potential: $-67 \text{ mV}$).
CORTICOTHALAMIC RESPONSES IN THALAMIC NEURONS

FIG. 13. Input-output curve plotting stimulus intensity vs. amplitude of corticothalamic-stimulation-evoked IPSPs. a: traces illustrating IPSPs evoked by stimulation of varying amplitude of corticothalamic afferents in a VB neuron. Note that both components of the IPSP were always activated, and that there appeared to be stepwise increments in IPSP amplitudes with increasing stimulus strength. b: input-output plot of the responses depicted in A. Note that the amplitude of the IPSP exhibited stepwise increments in amplitude in response to gradually increasing corticothalamic stimulus intensities. Filled circles: early IPSP components. Filled squares: later IPSP components.

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