# Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors

(thalamus/cerebral cortex/arousal)

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ABSTRACT     The mammalian thalamus forms an obliga-
tory relay for nearly all sensory information that reaches the
cerebral cortex. The transmission of sensory information by the
thalamus varies in a state-dependent manner, such that during
slow wave sleep or drowsiness thalamic responsiveness is
markedly reduced, whereas during the waking, attentive state
transmission is enhanced. Although activation of brainstem
inputs to thalamic neurons has long been assumed to underlie
this gating of sensory transfer through the thalamus, numer-
ically the largest input to thalamic relay neurons derives from
layer VI cells of the cerebral cortex. Here we report that
activation of corticothalamic fibers causes a prolonged excita-
tory postsynaptic potential in guinea pig dorsal lateral genic-
ulate relay neurons resulting from the reduction of a potassium
conductance, consistent with the activation of glutamatergic
"metabotropic" receptors. This slow depolarization can switch
firing of thalamic neurons from the burst firing mode, which
is prevalent during slow wave sleep, to the single spike mode,
which is prevalent during waking, thereby facilitating trans-
mission of sensory information through the thalamus. This
prolonged enhancement of thalamic transfer may allow the
cerebral cortex to gate or control selective fields of sensory
inputs in a manner that facilitates arousal, attention, and
cognition.

The transmission of sensory/motor information through the
thalamus to the cerebral cortex varies in a state-dependent
manner, such that transmission is degraded during periods of
slow wave sleep and enhanced during periods of arousal and
attentiveness (1–3). These alterations in thalamic excitability
are associated with depolarization of thalamic relay neurons
and reduction of certain types of intrathalamic inhibitory
potentials (4–6). These changes in thalamic excitability have
long been assumed to occur in response to increased activity
in ascending activating systems located in the brainstem and
hypothalamus (ref. 7; for reviews, see refs. 8 and 9). How-
ever, the major input to thalamic relay cells is from layer VI
cells of the cerebral cortex (10, 11). Physiological, pharma-
cological, biochemical, and immunocytochemical investiga-
tions suggest that this corticothalamic projection may use
glutamate as a neurotransmitter (12–15). Electrical stimula-
tion of the corticothalamic projection results in monosynaptic
excitation of thalamic relay neurons through typical excita-
tory amino acid "ionotropic" receptors (16, 17), which can
be followed by a prolonged (up to 30 sec) increase in neuronal
excitability (16). Although the cellular mechanisms of this
prolonged increase in excitability are not yet known, recent
*in situ* hybridization for the glutamate "metabotropic" re-
ceptor reveals neurons in the thalamus to strongly express
the message for this receptor (18). Because activation of
glutamate metabotropic receptors in hippocampal CA3 py-

ramidal cells causes slow excitation through a decrease in $K^+$
conductances (19, 20), we hypothesized that similar mecha-
nisms may account for slow corticothalamic modulation of
thalamic excitability. Here we show that the corticothalamic
projection controls the firing mode and excitability of tha-
lamic relay cells through a decrease in resting $K^+$ conduc-
tance, consistent with activation of the glutamate metabo-
tropic subclass of receptor.

## METHODS

Male or female guinea pigs were deeply anesthetized with
sodium pentobarbital (35 mg/kg i.p.) and killed by decapita-
tion, as described (21, 22). The thalamus was rapidly dis-
sected free and was sectioned either in the plane of the optic
tract fibers entering the dorsal lateral geniculate nucleus
(LGNd) or sagittally (in the plane of the corticothalamic
fibers) on a Vibratome as 400-$\mu$M slices. Slices were main-
tained in an interface style chamber at 36 ± 1°C and bathed
with a solution containing 126 mM NaCl, 2.5 mM KCl, 2 mM
$MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4$, 2 mM $CaCl_2$,
and 10 mM glucose, saturated with 95% $O_2$/5% $CO_2$ to final
pH 7.4. Agonists were applied locally with the "pico-drop"
method in volumes of 5–20 pl (21, 22). Antagonists were
applied in the bathing medium. To block responses to sero-
toninergic, $\alpha_1$-adrenergic, $H_1$-histaminergic, and muscarinic
receptors (9, 21–23), all experiments were conducted in the
presence of 1 $\mu$M methysergide, 1 $\mu$M prazosin, 1 $\mu$M
pyrilamine, and 1 $\mu$M scopolamine. Electrical stimuli (5 $\mu$A
to 1 mA; 50- or 100-$\mu$sec duration) were delivered through a
concentric bipolar stimulating electrode placed in either the
corticothalamic or optic tracts. The frequency of stimulation
was typically 50 Hz, although the slow excitatory postsyn-
aptic potential (EPSP) was present with frequencies ranging
from 3 to 500 Hz (data not shown). During tract stimulation,
the stimulating electrode was typically placed 750–2000 $\mu$m
from the border of the LGNd to avoid activation of nontract
fibers. No effort was made to remove the thalamic reticular
nucleus from sagittal slices. Intracellular recording micro-
electrodes were filled with 4 M potassium acetate and had a
final resistance of 40–55 MΩ. Single electrode voltage clamp
was done as described (23) while continuously monitoring
headstage output. Only neurons with stable membrane po-
tentials negative to −60 mV and input impedances >30 MΩ
were included in the present study. The precise course of the
corticothalamic projection from primary visual cortex to the
LGNd of the guinea pig was investigated through the anter-
ograde transport of biocytin and visualization with standard
avidin–biotin immunohistochemical techniques (24).

Abbreviations: LGNd, dorsal lateral geniculate nucleus; $[Ca^{2+}]_o$,
extracellular $Ca^{2+}$ concentration; $[K^+]_o$, extracellular $K^+$ concen-
tration; EPSP, excitatory postsynaptic potential; APCD, (1$S$,3$R$)-1-
aminocyclopentane-1,3-dicarboxylic acid; $I$–$V$, current vs. voltage.

## RESULTS

Electrical stimulation of corticothalamic fibers anterior to the guinea pig LGNd resulted in typical monosynaptic EPSPs in LGNd neurons, followed by inhibitory postsynaptic potentials as described (16, 17) both *in vivo* and *in vitro*. Interestingly, the delivery of two or more electrical stimuli at frequencies between 3 and 500 Hz (typically 50 Hz) caused a slow excitatory potential that lasted from 3 to 4 sec after two shocks (Fig. 1A, stimuli 2) up to >20 sec after a train of 10 stimuli (Fig. 1A, stimuli 10). This slow depolarizing response represents a slow EPSP because it is abolished by block of synaptic transmission either by reducing extracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_o$) to 0.5 mM and raising extracellular $[Mg^{2+}]$ to 8 mM or by local application (10 $\mu$M in micropipette) of the $Na^+$ channel poison tetrodotoxin ($n = 4$; data not shown). Incrementing the intensity of the train of stimuli revealed that the fast and slow EPSPs both exhibited similar thresholds (50–100 $\mu$A), and both increment gradually with increments in intensity of stimulation ($n = 20$). In addition, the fast EPSPs showed marked facilitation during the stim-

ulus train, a known property of EPSPs activated by corticothalamic fibers (16). In contrast, electrical stimulation of optic tract axons activated fast EPSPs that exhibited a clear threshold (between 10 and 20 $\mu$A) for activation of a major component of the EPSP, did not show facilitation during a stimulus train, and were never followed by a slow EPSP ($n = 5$; Fig. 1E).

The slow EPSP activated by stimulation in the corticothalamic tract was associated with a marked decrease in apparent input conductance (Fig. 1B), suggesting a possible underlying decrease in membrane conductance. We have previously demonstrated similar responses to activation of muscarinic, $\alpha_1$-adrenergic, and $H_1$-histaminergic receptors in thalamic relay neurons (9, 21–23). However, block of all of these receptors, plus serotoninergic receptors, with 1 $\mu$M methysergide, 1 $\mu$M scopolamine, 1 $\mu$M prazosin, and 1 $\mu$M pyrilamine failed to block the slow EPSP (Fig. 1; $n = 20$). Similarly, bath application of the quisqualate ionotropic antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5–30 $\mu$M), the $N$-methyl-D-aspartate (NMDA) receptor antagonist DL-amino-5-phosphonovalerate (APV; 50–100 $\mu$M)
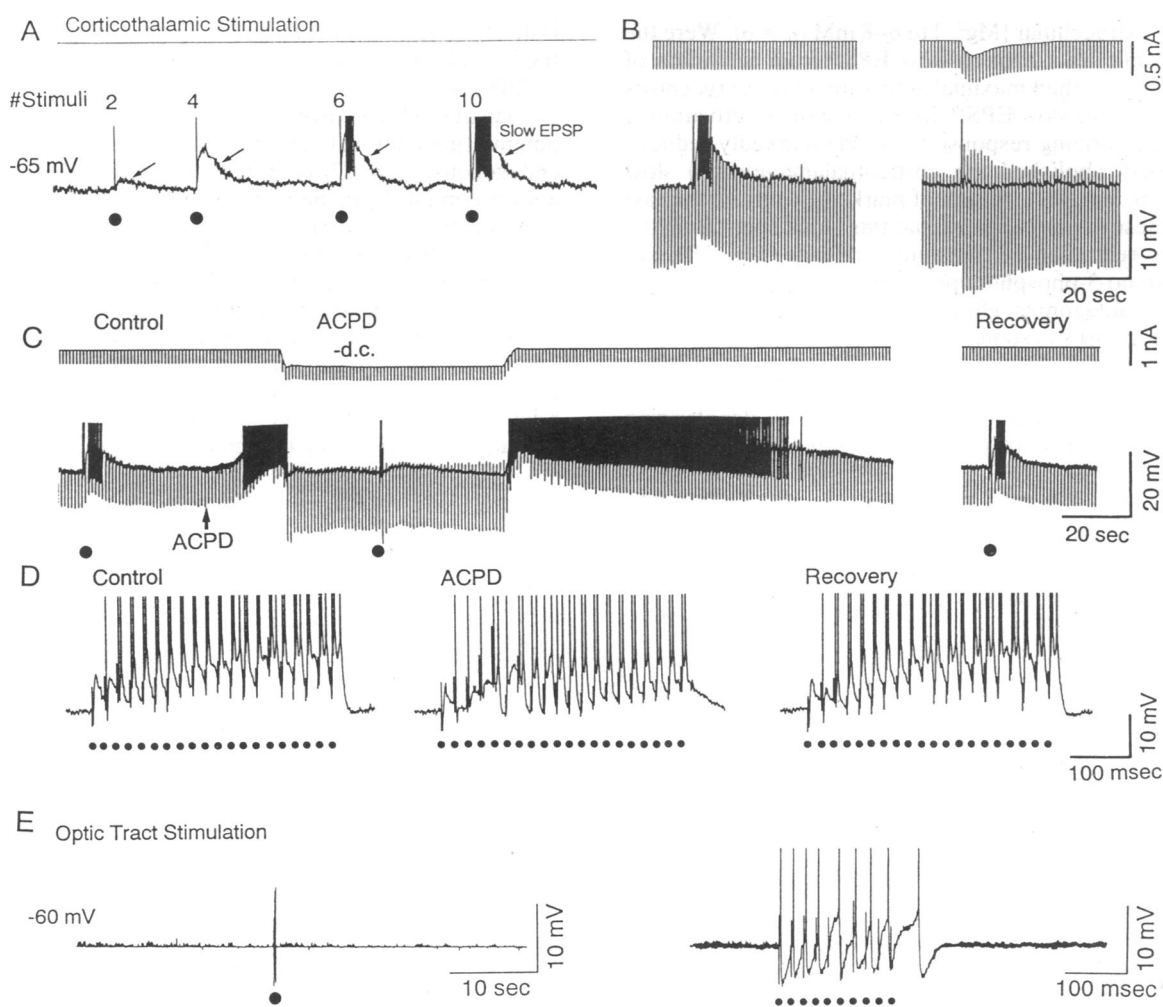


FIG. 1. Characteristics of the slow EPSP evoked by stimulation (black dots) of corticothalamic fibers. (A) Intracellular recording from a guinea pig LGNd neuron maintained in a sagittal slice *in vitro* during delivery of electrical shocks to the corticothalamic tract. As few as two shocks (0.17 mA, 50 Hz) generates a slow EPSP. Increasing the number of shocks in the train from 2 to 4, 6, and 10 increases amplitude and duration of the slow EPSP. (B) Compensation for the slow EPSP with intracellular injection of current illustrates that the slow EPSP is associated with a decrease in apparent input conductance. (C) Local application of a maximal dose of the glutamate metabotropic receptor agonist 1S,3R-1-aminocylopentane-1,3-dicarboxylic acid (ACPD) (400 $\mu$M in micropipette) causes a large slow depolarization. Compensation for this depolarization with intracellular injection of current [−direct current (d.c.)] reveals a substantial decrease in apparent input conductance. Corticothalamic stimulation now results in normal fast EPSPs (see D), while the slow EPSP is markedly reduced, indicating occlusion. This effect is fully reversible (Recovery). (D) Train of fast EPSPs generated by the electrical stimulation before, during, and after recovery from ACPD application. (E) Train of 10 electrical stimuli to the optic tract causes fast EPSPs and action potentials (right trace) but no slow EPSP (left trace). Data in A–D are from the same LGNd cell.

and the $\gamma$-aminobutyric acid type A (GABA$_A$) antagonist picrotoxin (10 $\mu$M) blocked the fast EPSP and inhibitory postsynaptic potentials but did not affect the slow EPSP or the response to the metabotropic receptor agonist ACPD ($n$ = 5; see below). Recently activation of glutamate metabotropic receptors in hippocampal pyramidal cells has been reported (19, 20) to result in slow depolarizing responses from reduction of the voltage or calcium-sensitive potassium currents known as $I_{AHP}$ and $I_M$. In situ hybridization techniques reveal that a large proportion of neurons in the thalamus stain for mRNA for the glutamate metabotropic receptor (18), and immunocytochemical, retrograde transport, and biochemical studies have implicated glutamate as a neurotransmitter in the corticothalamic projection (12–15). Consistent with the presence of glutamate metabotropic receptors on thalamic relay neurons, application of the specific glutamate metabotropic receptor agonist ACPD (100–400 $\mu$M in micropipette) to LGNd cells caused robust slow depolarizing responses associated with a marked decrease in input conductance (Fig. 1C). This response is a direct postsynaptic response because it persists after blocking synaptic transmission by local application of tetrodotoxin or by lowering [Ca$^{2+}$]$_o$ to 0.5 mM and raising extracellular [Mg$^{2+}$] to 6–8 mM ($n$ = 6). Were the corticothalamic tract-evoked slow EPSP from activation of ACPD receptors, then maximal activation of these receptors should occlude the slow EPSP. Indeed, maximal activation of the slow depolarizing response to ACPD markedly reduced or completely abolished the corticothalamic-evoked slow EPSP ($n$ = 6; Fig. 1C). A lack of marked effects on the fast EPSPs at these doses indicates that this block is not from the presynaptic reduction of transmitter release (Fig. 1D). Although 2-amino-3-phosphonoproprionic acid (AP-3) has been reported to antagonize glutamate metabotropic receptor-mediated responses in some neuronal systems (25), we found this substance ineffective in blocking responses to ACPD in LGNd neurons.

The ionic mechanisms underlying the slow depolarizing response to activation of glutamate metabotropic receptors

were analyzed with single electrode voltage-clamp techniques. Application of ACPD resulted in an inward current that was associated with a decrease in apparent input conductance at all membrane potentials and that reversed to an outward current at an average of −83 mV (± 1.6 mV; SEM; $n$ = 4) in 7.5 mM extracellular K$^+$ concentration ([K$^+$]$_o$) (Fig. 2A). Subtraction of the $I$-$V$ plot obtained during ACPD action from control $I$-$V$ relations revealed that the current suppressed by ACPD is relatively voltage independent in the membrane potential range of −60 to −130 mV (Fig. 2 $D$ and $E$). Changing [K$^+$]$_o$ to 2.5 mM shifted the reversal potential of the ACPD response to an average of −108 mV (±3 mV; $n$ = 9), which is close to that predicted by the Nernst equation (−111 mV) (Fig. 2 $B$ and $E$). Changing [K$^+$]$_o$ back to 7.5 mM shifted the reversal potential back toward control levels (Fig. 2C). These results indicate that activation of the ACPD subtype of excitatory amino acid receptor in LGNd relay neurons reduces a K$^+$ current the conductance of which is not particularly voltage dependent and which contributes substantially to the resting "leak" conductance of the cell, leading us to term this current $I_{Kleak}$. In contrast to cortical pyramidal cells (28), LGNd relay neurons appear to possess little of the K$^+$ currents $I_M$ and $I_{AHP}$ (29), which may explain the lack of prominent voltage dependence in the response to ACPD (19).

Thalamic relay neurons display two basic forms of action potential generation: ($i$) rhythmic burst firing generated either endogenously (26, 27) or in response to inhibitory postsynaptic potentials from the $\gamma$-amino-butyric acid-releasing cells of the nucleus reticularis (4), or ($ii$) single spike activity (4, 5). The switch from rhythmic burst firing to single spike activity is associated with awakening and increases in electroencephalogram (EEG) desynchronization and is achieved through depolarization of the membrane potential by 10–20 mV (4, 5). That activation of glutamate metabotropic receptors may cause a similar change in firing mode was examined in LGNd relay neurons. Application of ACPD or stimulation of corticothalamic fibers during the injection of hyperpolarizing and
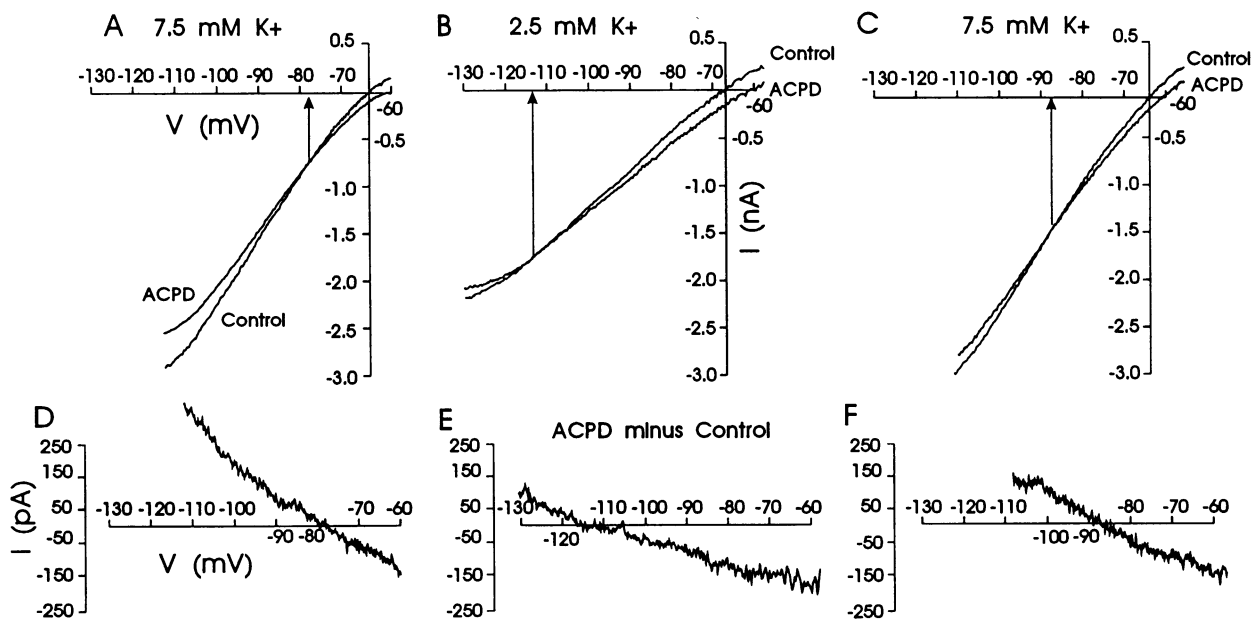


FIG. 2. Response of thalamic neurons to the glutamate metabotropic receptor agonist ACPD and the ionic basis for this response. (A) Current vs. voltage ($I$-$V$) relation of a guinea pig LGNd neuron before and during ACPD action. ACPD results in a marked inward current and decrease in apparent input conductance. This response reverses to an outward current at −78 mV. Changing [K$^+$]$_o$ from 7.5 to 2.5 mM results in a shift of the reversal potential to −114 mV (B), whereas shifting back to 7.5 mM [K$^+$]$_o$ shifts the reversal potential back toward control (−88 mV) (C). Subtraction of the control $I$-$V$ relation from that during ACPD reveals that the current suppressed by ACPD varies in a relatively linear manner with membrane potential (D–F). $I$-$V$ relations were obtained after block of the hyperpolarization-activated cation current $I_h$ with local application of Cs$^+$ (10 mM in micropipette), as described (26, 27).

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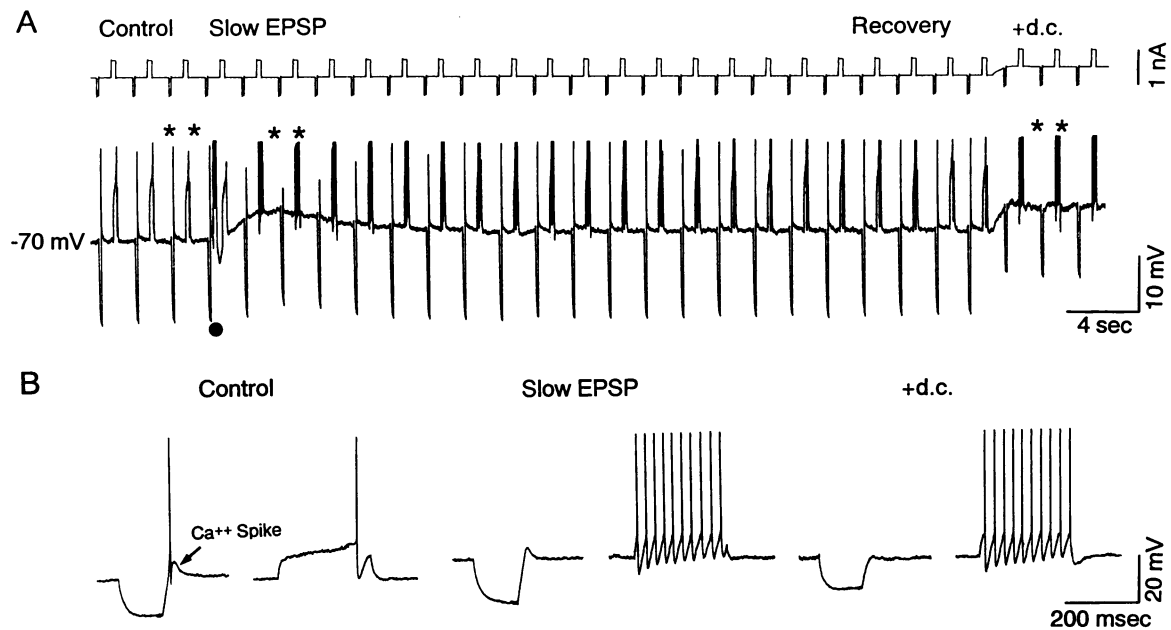*Proc. Natl. Acad. Sci. USA 89 (1992)*    2777



FIG. 3. The slow EPSP suppresses rebound burst firing and promotes single spike activity. (*A*) Intracellular injection of a hyperpolarizing current pulse in a LGNd cell results in a rebound low threshold $Ca^{2+}$ spike, whereas injection of a depolarizing current pulse causes a single action potential (*A* and *B*, Control). Electrical activation of corticothalamic fibers (10 stimuli, 50 Hz, 0.3 mA) results in a slow EPSP that abolishes the rebound response to the hyperpolarizing input and greatly increases the number of action potentials generated in response to the depolarizing current pulse (*B*, slow EPSP). This effect is due to the changes in membrane potential because neuron depolarization by a similar amount with the intracellular injection of current causes a similar shift in firing mode [*A* and *B*, +direct current (d.c.)]. Asterisks indicate traces expanded for detail in *B*.

depolarizing current pulses markedly inhibited the ability of the hyperpolarizing pulses to generate rebound low threshold $Ca^{2+}$ spikes (Fig. 3) and greatly facilitated the ability of depolarizing current pulses to generate trains of action potentials (Fig. 3; $n = 7$). These alterations in firing mode appear to occur largely through membrane depolarization because depolarization by an equal amount with the intracellular injection of current had a similar effect (Fig. 3, +direct current). This result indicates that the firing mode of thalamic relay neurons may be controlled by descending corticothalamic axons through the activation of glutamate metabotropic receptors.

## DISCUSSION

Numerically corticothalamic fibers form the major synaptic input to thalamic relay neurons, suggesting that this pathway has an important function in the regulation of thalamocortical activity (10, 11). Although numerous investigators have proposed (30–33) that the corticothalamic pathway may facilitate synaptic transfer of sensory and motor information through the thalamus by monosynaptic excitation of relay neurons, a specific mechanism for the long-lasting facilitation of thalamic excitability that may occur in response to corticothalamic stimulation has been lacking (16). Here we demonstrate that activation of corticothalamic fibers results in a slow depolarization of thalamic relay cells through reduction of a potassium current, presumably through the activation of glutamate metabotropic receptors. This slow depolarization blocks rebound burst firing and promotes single spike activity, thereby promoting a state of thalamic activity that is associated with enhanced sensory transmission, arousal, and cognition (1–6). The point-to-point anatomical specificity of the corticothalamic projection indicates that this pathway may be capable of modulating thalamic activity in a manner precise enough to enhance transfer through specific sensory modalities or even to specific locations in sensory space in accordance with behavioral demands. The temporally pro-

longed nature of the corticothalamic slow EPSP suggests that this potential is probably involved in behavioral state changes that occur on the time scale of seconds or longer, whereas the ionotropic receptor-mediated fast corticothalamic EPSPs may be more important for more phasic modulation of thalamic excitability. The present results challenge the notion that activation of thalamocortical circuits arises *exclusively* in lower lying systems, such as those in the brainstem (7), hypothalamus (23, 34), and basal forebrain (35) because the "descending activating system" formed by the corticothalamic pathway is itself capable of modulating the pattern of activity in thalamocortical systems through precisely the same ionic mechanisms that have been associated with the ascending brainstem and hypothalamic activating systems (9, 23–27). Indeed, the present results, and those of others (19, 20), suggest that the shifting of neuronal circuits from a state associated with drowsiness or sleep to one associated with arousal may be a consequence of increased activity in the circuit itself, in addition to the actions of modulatory transmitters released from neurons located in lower lying brain structures (for review, see ref. 8). The existence of such intrinsic and descending activating mechanisms in thalamocortical systems may explain such diverse phenomenon as the persistence of arousal during progressive and massive lesions of the brainstem (36), recovery from brainstem lesion-induced coma (37), and the ability to consciously modulate ones own level of arousal.

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