Corticothalamic Inhibition in the Thalamic Reticular Nucleus

Liming Zhang and Edward G. Jones

Center for Neuroscience, University of California, Davis, California 95616

Submitted 1 July 2003; accepted in final form 20 October 2003

Zhang, Liming and Edward G. Jones. Corticothalamic inhibition in the thalamic reticular nucleus. J Neurophysiol 91: 759–766, 2004. First published October 29, 2003; 10.1152/jn.00624.2003. Mutual inhibition between the GABAAergic cells of the thalamic reticular nucleus (RTN) is important in regulating oscillations in the thalamocortical network, promoting those in the spindle range of frequencies over those at lower frequencies. Excitatory inputs to the RTN from the cerebral cortex are numerically large and excitatory powerful in inducing spindles. However, the extent to which corticothalamic influences can engage the inhibitory network of the RTN has not been fully explored. Focal electrical stimulation of layer VI in the barrel cortex of the mouse thalamocortical slice in vitro resulted in prominent di- or polysynaptic inhibitory postsynaptic currents (IPSCs) in RTN cells under the experimental conditions used. The majority of cortically induced responses consisted of mixed PSCs in which the inhibitory component predominated or of large IPSCs alone, implying inhibition of neighboring cells other, cortically excited RTN cells. Within the mixed PSCs, fixed and variable latency components could commonly be identified. IPSCs could be blocked by application of ionotropic glutamate receptor antagonists or of GABAA receptor antagonists, also indicating their dependence on corticothalamic excitation. Disynaptic or polysynaptic inhibition. Spontaneous GABAA receptor-dependent IPSCs were routinely observed in the RTN and, taken together with the results of cortical stimulation, indicate the existence of a substantial network of intrareticular inhibitory connections that can be effectively recruited by the corticothalamic system. These results suggest activation of cortical excitatory inputs triggers the propagation of inhibitory currents within the RTN and support the view that activation of the RTN from the somatosensory cortex, although focused by the topography of the corticothalamic projection, is capable of disynaptically engaging the whole inhibitory network of the RTN, by local and probably by reentrant GABAA receptor–based synapses, thus spreading the corticothalamic influence throughout the RTN.

INTRODUCTION

The thalamic reticular nucleus (RTN) is a sheet of GABAAergic neurons that covers the external surface of the thalamus. In rodents and certain other species it forms the principal source of inhibition to the relay neurons of most dorsal thalamic nuclei (reviewed in Steriade et al. 1997). GABAAergic synapses form ~30% of the synapses on rodent RTN cells (Liu and Jones 1999) and are mostly formed by the intranuclear collaterals of axons of RTN cells that project into the dorsal thalamus (Cox et al. 1996; Deschênes et al. 1985; Liu et al. 1995; Pinault et al. 1995a,b, 1997; Pinault and Deschênes 1998; Yen et al. 1985). In other species, there are also GABAAergic dendrodendritic synapses between RTN cells in cats (Deschênes et al. 1985; Yen et al. 1985), but in monkeys they are described as common in some studies and rare in others (Asanuma 1994; Williamson et al. 1994). In rodents, their existence is debated (de Biasi et al. 1986; Pinault et al. 1997).

It is thought that the capacity of RTN neurons to inhibit one another is a key element in preventing the occurrence of hypersynchronized, low-frequency oscillations of the thalamocortical network that underlie certain forms of seizures (Huguenard and Prince 1994; Huntsman et al. 1999; McCormick and Bal 1997; Sohal et al. 2000; von Krosigk et al. 1993). The inhibitory interactions serve to dampen the recurrent excitation of RTN cells by bursting relay cells subjected to RTN-based inhibition that underlies oscillations in the spindle range of frequencies (Bal et al. 1995a,b; von Krosigk et al. 1993; Warren et al. 1994). Evidence for functional inhibitory synapses between RTN cells has been obtained in experiments in which the RTN was subjected to focal electrical stimulation in vitro (Huntsman et al. 1999; Huntsman and Huguenard 2000; Zhang et al. 1997) or in vivo (Bazhenov et al. 1999) or in which RTN or perigeniculate neurons were directly stimulated by local application of glutamate in vitro (Sanchez-Vives et al. 1997a; Shu and McCormick 2002). None of these studies, however, has addressed the issues of the capacity of extrinsic sources of input to induce inhibition in the RTN nor the extent of spread of this inhibition within the RTN.

The corticothalamic projection forms one of the principal sources of extrinsic input to the RTN. Corticofugal synapses form more than 60% of the synapses on rodent RTN neurons (Liu and Jones 1999) and are capable of powerfully exciting these neurons (Golshani et al. 2001; Liu et al. 2001). Corticofugal stimulation is a potent initiator of spindle oscillations in vivo and in vitro (Steriade et al. 1972; Warren et al. 1994), serving to recruit large-scale ensembles of synchronously firing RTN and relay cells into spindle activity. Yet the collaterals of corticofugal axons that innervate the RTN in rodents terminate in highly focal domains that can influence directly only a limited number of RTN cells (e.g., Agmon et al. 1995; Bourassa et al. 1995). The present study attempts to determine the capacity of corticofugal inputs to elicit inhibitory events in RTN neurons and the potential for this inhibitory influence to spread through the RTN.

METHODS

Preparation of in vitro slices

Postnatal day 2 (P2) to P17 ICR mice (Harlan Sprague–Dawley, Indianapolis, IN) were anesthetized by hypothermia (P2–P4) or with ether (P5–P17) and decapitated. The brain was quickly removed and put into chilled artificial cerebrospinal fluid (ACSF), in which 126 mM NaCl had been replaced by sucrose at equivalent osmolarity and...
containing (in mM) 252 sucrose, 3 KCl, 1.25 NaH₂PO₄, 1.3 MgSO₄, 7 H₂O, 2.5 CaCl₂, 26 NaHCO₃, and 10 dextrose, pH 7.4 when bubbled with 95% O₂-5% CO₂, osmolarity 300–315 mOsm. Slices (400 μm thick) containing the somatosensory cortex, RTN, and ventral posterior nucleus (VP) were cut at an angle that preserved corticothalamic and thalamocortical connectivity (Agmon and Connors 1991). The slices were cut on a vibratome while immersed in chilled ACSF and were then transferred to a submerged-type recording chamber. The slices were adjusted to 7.3 with KOH, and final osmolarity was 290–310 mOsm/kg. Series resistance during recordings was regularly 5–25 MΩ.

For voltage-clamp experiments, QX314 (5 mM) was routinely added to the internal solution to prevent voltage-sensitive Na⁺ channels from generating action potentials. The resting membrane potential (RMP) was measured just after rupturing the cell membrane and in most of the experiments the cells were held at −70 mV. Data were low-pass-filtered at 2–10 KHz and digitized at 1–10 KHz via a CED 1401 plus interface (Cambridge Electronic Design) with a Pentium-based computer (Gateway 2000) that stored the data and provided on-line display of responses and off-line data analysis. CED patch- and voltage-clamp software was used to acquire and analyze data. Spontaneous and evoked postsynaptic currents (PSCs) were also analyzed with MiniAnalysis 5.1.1 (Synaptosoft). The liquid junction potential error was not corrected. Plots were made using Microcal Origin 5.0 (Microcal Software) and Sigmaplot 2000 (SPSS).

Recording

The RTN was visualized with differential interference contrast and infrared optics (Fig. 1B). Whole cell patch-clamp recordings were performed using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Recording pipettes were pulled from borosilicate glass on a Narishige PP-83 two-stage puller. In the experiments, pipettes had resistances of 3–5 MΩ when filled with internal solutions containing (in mM) 130 potassium gluconate, 10 KCl, 2 MgCl₂, 0.1 CaCl₂, 1.1 EGTA, 10 HEPES, 2 K₂-ATP, and 0.5 GTP. The pH was adjusted to 7.3 with KOH, and final osmolarity was 290–310 mOsm/kg.

Synaptic stimulation

Corticothalamic synaptic responses were evoked in RTN neurons by electrical stimulation of layer VI of the somatosensory cortex, using 0.1 ms cathodal stimuli delivered at 0.07 Hz to a single site in layer VI via a monopolar tungsten microelectrode (Fig. 1A). Stimulus strength was set 10–15% above absolute threshold to elicit a stable response.

Morphology

Biocytin 0.5% was present in the internal solution of the recording pipette during all recordings. Slices were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, incubated in avidin—biotin—peroxidase complex (ABC kit PK-4000, Vector Laboratories) and reacted with 3,3'-diaminobenzidine—HCl to visualize injected cells, which were then drawn with the aid of a camera lucida (Fig. 1C).

Drug application

The following agents were applied through the superfusing ACSF: 6-cyano-7-nitroquinolinic acid (CNQX); (+)-2-amino-5-phosphonoentanoic acid (APV), and (+)-bicuculline methiodide (BMI); all were obtained from Tocris Cookson (Bristol, UK). These drugs were made up as 10 mM stock solutions in distilled water and diluted with ACSF to the final concentration just before addition to the perfusion medium. The effects of the antagonists were continuously recorded and the drugs were later washed out by perfusion with normal ACSF for 20–40 min or longer until predrug application responses returned.

Statistics and measurement of synaptic responses

Amplitudes of whole cell PSCs were measured as the difference between the average of 0.5-ms regions spanning the baseline immediately before the onset of the PSC and those straddling the peak of the PSC. Noise was determined by measuring the difference of means of 0.5-ms regions spanning the baseline region and a region separated by the same interval separating baseline and PSC-peak regions. The threshold for measurement for the amplitudes of spontaneous PSCs was limited to 10 pA by using Mini-Analysis software. Data are presented as the means ± SE. Student’s t-test was used to compare two means unless otherwise stated and a P < 0.05 was required for statistical significance using Microcal Origin 5.0.

RESULTS

During whole cell recording, biocytin 0.5% was present in the internal solution of the recording pipette. Injection of biocytin into neurons in the somatosensory sector of the RTN showed the typical dendritic morphology of RTN cells and the primary axon that entered and ramified focally in zones pre-

![Figure 1](http://jn.physiology.org/)
sumably corresponding to barreloids in the underlying VP nucleus at all ages. It also revealed the presence of one to two collaterals given off before the primary axon left the RTN. These collaterals extended for no more than 100–200 μm (mean approximately 150 μm; n = 4) through the RTN, usually within the dendritic field of the parent cell and without further branching (Fig. 1C).

In general, electrophysiological properties of the RTN cells showed that average RMP was −60 mV and average threshold potential was −48 mV. The cells showed typical tonic and burst behavior when injected with small depolarizing or hyperpolarizing current pulses at different membrane potentials (Fig. 1D). A train of tonic discharges was elicited at membrane potentials positive to approximately −55 mV while repetitive low threshold spikes and burst discharges were elicited at membrane potentials negative to approximately −55 mV (Fig. 1D). The mean rebound burst frequency in response to injecting −200 pA currents was 2 Hz under the experimental conditions used.

**Spontaneous GABA\textsubscript{A} receptor-based IPSCs in the RTN**

Under resting conditions in the slice, neurons recorded in the somatosensory sector of the RTN (n = 6) with membrane potentials held at −70 mV exhibited inward spontaneous inhibitory PSCs (IPSCs; Fig. 2A). The majority of these had amplitudes of 10–20 pA (mean, 14.14 ± 0.2) or 20–60 pA (mean, 42.8 ± 4.0), suggesting the presence of unitary events at the lower amplitudes and combined events at the higher amplitudes (Fig. 2B). All spontaneous responses were completely abolished by bath application of 10 μM BMI (Fig. 2A), indicating their dependence on GABA\textsubscript{A} receptor-based mechanisms.

**Cortical stimulation evokes predominant IPSC/inhibitory postsynaptic potentials (IPSPs) in the RTN**

Low-intensity electrical stimuli applied focally to the cells of origin of corticothalamic fibers in layer VI of the somatosensory cortex evoke excitatory PSCs (EPSCs) at two distinct latencies in RTN cells of the mouse thalamocortical slice (Golshani et al. 2001; Liu et al. 2001). EPSCs with latencies in the range of 2 to 4 ms are due to antidromic activation of thalamocortical fibers and orthodromic invasion of their collaterals in the RTN. EPSCs with latencies in the range of 6 to 9 ms are due to orthodromic activation of slower conducting corticothalamic fibers and their collaterals in the RTN. For the purposes of the present study, all responses with latencies < 6 ms were excluded.

Cortical stimulation will elicit N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated excitatory postsynaptic potentials (EPSPs)/EPSCs in RTN cells of the mouse thalamocortical slice but these EPSPs/EPSCs are significantly attenuated in the presence of GABA\textsubscript{A} receptor-mediated inhibition (Warren and Jones 1997). In the present study, under the recording conditions used, pure EPSPs/EPSCs were rarely observed. In recordings from 51 RTN neurons showing responses to low-frequency electrical stimulation of layer VI, the typical evoked PSC consisted of either single or multiple peaked components with fixed or variable latencies (defined below) and with a distinctive slow decay (Fig. 3A). The majority of PSCs consisted of mixed IPSCs and EPSCs with a predominant IPSC component (34 neurons) and the remaining responses (17 neurons) consisted of pure IPSCs. Responses were not confined to a localized focus in the RTN and could be obtained at recording sites as distant as approximately 1 mm from one another (Fig. 1B).

All the evoked PSCs recorded in RTN neurons in response to cortical stimulation could be either fully or partially removed by 10 μM BMI (Figs. 3, A, C, and E and 4A). PSCs recorded in 17 of 51 neurons were completely blocked by 10 μM BMI, indicating that these PSCs were GABA\textsubscript{A} receptor-mediated IPSCs, and no EPSC component was found at several holding membrane potential levels ranging from −100 to +20 mV (increment 20 mV) in these cells (Fig. 3, A and C). In the remaining 34 neurons, cortically evoked PSCs could be strongly attenuated by BMI (Figs. 3E and 4A) and the residual currents could be cleared by adding CNQX and APV to the bath solution. In some cases (n = 5), EPSCs were masked by IPSCs and could only be revealed when membrane potentials were held at depolarized levels (−20 mV) when the reversed polarity of PSCs from outward IPSC to inward EPSC became clear in the presence of BMI (Fig. 3E). The similar reversed polarity was also observed in current-clamp recording (Fig. 4A). These EPSCs/EPSPs unmasked from predominant IPSC/EPSPs by application of the GABA\textsubscript{A} receptor antagonist exhibited characteristics that indicated a strong NMDA receptor-
mediated component with a slow decay time and were substantially reduced by APV (50 µM, data not shown).

Cortically evoked PSCs were also sensitive to application of the glutamate receptor antagonists CNQX and APV and the response could be completely abolished by bath application of 20 µM CNQX and 50 µM APV (Figs. 3C and 4, A and C), as readily as with 10 µM BMI. Seventy percent of cells showing pure IPSC/IPSPs (5/7) were also equally sensitive to BMI or CNQX/APV, indicating the dependence of the responses on monosynaptic corticothalamic excitation of other RTN cells followed by di- or polysynaptic inhibition. IPSCs removed by CNQX and APV were characterized by a rapid, “all-or-none” disappearance in about 90 s, and recovered with a gradually shortened latency in a very slow manner after washing (Fig. 4, C and D), suggesting that the key factor in initiating an inhibitory chain reaction in the RTN is the monosynaptic, excitatory corticothalamic synapse. This result clearly indicates that RTN cells excited by glutamatergic corticothalamic collateral synapses are closely coupled to their neighbors by GABA A receptors—containing inhibitory synapses.
Corticothalamic stimulation elicits postsynaptic responses with fixed and variable latencies in RTN neurons

The PSCs recorded in response to stimulation of layer VI commonly showed complex, multipeaked wave forms in which early, relatively fixed, and later, variable latency components could be identified (Figs. 3A–C and 4, A and C). We shall define a fixed latency response as a response in which, on a trial-by-trial basis, the variance between individual responses was <1 ms. On this basis, 36 of 51 neurons showed a fixed latency response with some of them followed by a second response of variable latency. In Fig. 3A, the evoked response consisted of a small PSC with a fixed latency of 7.6 ms, followed by a second large PSC with a variable latency (range 35.6–48.6 ms; mean 42.3 ± 1.7 ms, n = 8 traces). The remaining 15 cells showed only variable response latencies ranging from two more or less consistent responses to responses with widely differing latencies (Fig. 4A). To determine the range and accuracy of the timed responses, we examined the mean and SD of the PSCs with variable latencies. The latencies of the 15 cells shown in Fig. 5B ranged from 9 to 92 ms with a mean of 32 ± 5.9 ms. There was a correlation between the mean latency and SD (r = 0.82, P = 0.0001; Fig. 5B), indicating that accuracy of the timed response decreases as the interval increases.

In cells with fixed response latencies, as defined above, the mean latency of this response was 9.4 (±2.1 ms; n = 36; Fig. 5A). The mean latency was significantly shorter in these neurons than in those with variable latencies of response (Fig. 5C; independent t-test, P < 0.0001). The PSCs with the shorter fixed latencies usually presented a single peak (Fig. 3, C and E), suggesting that PSCs with shorter latencies are largely mediated by disynaptic transmission following monosynaptic cortical excitation of neighboring RTN cells. Responses with fixed latencies did not show changes in latency in the presence of BMI. For 21 cells studied in detail, the latency in control conditions was 9.57 ± 0.4 ms, and in the presence of BMI it was 9.45 ± 0.4 ms. The slight difference was not statistically significant.

In 4 of 17 cells whose responses consisted of pure IPSCs (see above), the IPSC was of fixed latency. Pure IPSCs were observed in the majority (87%) of the neurons (13/15) that exhibited variable latencies of response. In cells with mixed IPSCs/IPSPs and EPSC/EPSPs, the mean latency became significantly shorter during the presence of BMI in the bath solution (Figs. 4A and 5C; 19.47 ± 2.93 ms in control: 10.89 ± 1.26 in BMI; Student’s t-test, P = 0.01, n = 5). After washout of BMI from bath solution, the latencies recovered to their original length (Fig. 4, A and C). This result suggests that, although dominant, the IPSC/IPSPs have longer latencies than the EPSP/IPSPs and are thus likely to be di- or polysynaptic; it further suggests that inhibitory activity driven by excitatory corticoreticular inputs propagates in the RTN.

Reversal potentials of the cortically evoked PSCs were examined in 23 neurons (Fig. 5, B and D). The mean reversal potential for the responses showing variable latencies was −50.1 ± 3.3 mV (range −34 to −69.8 mV; n = 10), whereas the mean reversal potential for fixed latency responses was −37.0 ± 6.1 mV (range 5 to −65 mV; n = 13). There was a significantly negative correlation between age and reversal potential in the responses with variable latencies (r = −0.73, P = 0.01, n = 10; Fig. 5D). The average reversal potential for PSCs with variable latencies (−50 mV) was closer to the calculated chloride equilibrium potential of −57 mV under these recording conditions, but there were 4 cells with reversal potentials less than −50 mV that were recorded in younger animals (less than P5). The average reversal potential for PSCs with fixed latencies (−37 mV) was 10 mV more positive than the PSCs with variable latencies, implying that the fixed latency PSC, although mixed, contained a substantial AMPA/NMDA receptor–mediated component (which alone would have a reversal potential of approximately 0 mV). These results indicate that reversal potential correlates negatively with developmental age for Cl−–based GABA A receptor–mediated inhibitory components. However, in the younger animals, reversal potentials might not match the chloride equilibrium potential. A more positive reversal potential might be attributable to a chloride uptake mechanism in the younger cells or, alternatively, to an increased permeability of the synaptic channel in the younger cells (Agnon et al. 1996; Ben-Ari et al. 1989; Zhang et al. 1991).

**Discussion**

This study reveals that electrical stimulation of layer VI of the somatosensory cortex, after excluding responses due to antidromic activation of thalamocortical axons, elicits PSCs in RTN cells that are dominated by inhibition. The currents elicited in some RTN cells had fixed latencies and were made...
up of an EPSC accompanied by a predominant IPSC. Currents elicited in many other RTN cells had variable latencies and were composed of IPSCs only. The results suggest that local excitation of RTN cells by focused corticothalamic terminations are capable of engaging the network of inhibitory connections throughout a large part of the RTN.

Most IPSCs evoked in RTN cells by cortical stimulation were multicomponent, with variable and often long latencies, temporal persistence, and fluctuations. These characteristics suggest that they were the result of polysynaptic propagation of activity through the circuitry of the RTN and in this they resembled the effects recorded in a number of other defined circuits excited polysynaptically (Agmon et al. 1996; Buonomano 2003; Kaneko et al. 2000; Metherate and Cruikshank 1999). Ando et al. (1995) had earlier shown latency fluctuation and the presence of temporal facilitation in the responses of RTN cells to cortical stimulation. Shortening of mean latency of PSCs/PSPs, as we found after IPSCs/IPSPs were abolished, is also a likely indication of polysynaptic transmission in the RTN. That is to say, polysynaptic spread of inhibition will normally last longer than an initial excitatory effect. Altered latencies of evoked responses or shortening of the duration of IPSP fluctuations in the presence of GABA_A receptor antagonists have also been reported in the cerebral cortex (Agmon et al. 1996; Buonomano 2003; Metherate and Cruikshank 1999).

Low-intensity electrical stimuli applied focally to the cells of origin of corticothalamic fibers in layer VI of the somatosensory cortex evokes EPSCs at two distinct latencies in RTN cells of the mouse thalamocortical slice (Golshani et al. 2001; Liu et al. 2001). EPSCs with latencies in the range of 2 to 4 ms are due to antidromic activation of thalamocortical fibers and orthodromic invasion of their collaterals in the RTN. EPSCs with latencies in the range of 6.5 to 9.5 ms are due to orthodromic activation of slower conducting corticothalamic fibers and their collaterals in the RTN. In the present study, only the longer latency, orthodromic responses were examined, although we cannot exclude that antidromic activation of other RTN cells could have contributed to longer latency inhibitory responses. The distance between the stimulating and recording sites was 1000 to 2000 μm, and assuming an axonal conduction velocity of approximately 0.2 mm/ms (Buonomano 2003; Tanifuji et al. 1994), the orthodromic latency of corticothalamic EPSCs in the RTN should be 5–10 ms, which is in the range derived by Golshani et al. (2001) and Liu et al. (2001). The mean latency of evoked PSCs with variable latencies was distinctly longer than that of evoked PSCs with fixed latencies. The longer latencies of the variable responses imply, again, that these longer latency IPSCs are the result of polysynaptic activation of the intrareticular network.

The presence of a small number of fixed latency IPSCs in the responses to cortical stimulation could indicate close coupling of the cell recorded from to a focus of corticothalamic fiber terminations that do not synapse directly on the cell, although it is conceivable that an excitatory input to the cell could have been severed by the slicing procedure. Morphological data show that the axonal collaterals of corticothalamic fibers in the RTN are relatively short and that the projection of a single-barrel-associated part of layer VI terminates in a very constrained focus approximately 50 μm in extent in the mouse RTN (Agmon et al. 1995). Hence, local stimulation in layer VI is likely to activate only a limited number of RTN cells and spread of activity within the RTN will be by inhibitory connections between RTN cells. There is also the possibility that GABA_A receptor–mediated currents are actually depolarizing under conditions in vivo, and spread activity directly through the RTN, eliciting burst discharges in the neurons and predisposing the network to spindle oscillations (Bazhenov et al. 1999).

GABA_A receptor–based inhibition has been demonstrated between RTN neurons, and it has now been shown that these synapses can be engaged by the corticothalamic projection. Inhibitory synapses between GABAergic cells have been demonstrated morphologically in several species (Deschênes et al. 1985; Liu and Jones 1999; Ohara and Lieberman 1985; Williamson et al. 1994; Yen et al. 1985) and physiologically in vitro in ferrets and rodents (Huntsman et al. 1999; Sanchez-Vives et al. 1997a,b; Shu and McCormick 2002; von Krosigk et al. 1993; Zhang et al. 1997) and in vivo in cats (Bazhenov et al. 1999). The consensus of most morphological studies is that most of the GABAergic synapses in the RTN are formed by the intranuclear collaterals of axons of the RTN cells. There is uncertainty about the extent to which dendrodendritic synapses between RTN cells are a feature of the RTN, since they have been described as extensive in some species such as cats (Deschênes et al. 1985; Yen et al. 1985), either common (Asanuma 1994) or rare (Williamson et al. 1994) in monkeys, and absent in rodents (Ohara and Lieberman 1985). Physiological studies based on whole cell or single-unit recordings in association with local electrical (Huntsman et al. 1999; Huntsman and Huguenard 2000; Zhang et al. 1997) or glutamate (Sanchez-Vives et al. 1997a,b; Shu and McCormick 2002) stimulation of RTN or perigeniculate GABAergic neurons, or made during network operations of the connected RTN and dorsal thalamus (Kim and McCormick 1998; Warren et al. 1994), have shown that GABA_A receptor–based inhibitory events can be readily identified as spontaneous events or after local stimulation of the RTN. GABA_B receptor–based effects in the RTN are relatively uncommon or difficult to elicit (Sanchez-Vives et al. 1997a; Ulrich and Huguenard 1996), a feature that probably reflects the low expression of GABA_B receptor gene expression in the RTN (Liang et al. 2000; Muñoz et al. 1998).

The present observations are in agreement with those of the previous studies in demonstrating the ubiquity of spontaneous and evoked GABA_A receptor–based inhibitory synaptic currents in RTN neurons. They demonstrate that the GABAergic synapses of the RTN can be engaged disynaptically, both locally and at a distance, by corticothalamic activation. These inhibitory interactions between RTN cells can operate over quite long distances within the RTN, as suggested by our recording of IPSCs in RTN cells as distant as 1 mm from one another. They should permit neurons in the somatosensory sector of the RTN to influence those in other sectors. Spread of inhibitory activity in the RTN could also be facilitated by the reentrant circuitry from the underlying VP nucleus. Burst discharges evoked in RTN cells by cortical stimulation will generate burst discharges in thalamocortical relay cells as they recover from RTN-based inhibition. The thalamocortical axon collaterals of these cells, in exciting RTN cells beyond the focus of original cortical excitation, would cause them to inhibit their neighbors, and so on in a manner akin to that originally proposed by Crabtree et al. (1998), thus also facili-
tating the spread of inhibitory activity in the RTN. The spread of hyperpolarizing inhibitory currents through the network of RTN neurons could also be potentiated by the presence of low-resistance gap junctions between the RTN cells (Landisman et al. 2002).

Previous studies that relied on local electrical stimulation of the RTN, although demonstrating the presence of GABA<sub>A</sub> receptor–based inhibition between RTN or perigeniculate neurons, could have stimulated diverse inputs to the RTN in addition to exciting the RTN cells directly. Studies involving local activation of RTN cells by glutamate did not reveal the extent of these inhibitory effects through the RTN. The present study stresses the efficacy of the corticothalamic projection in di- and polysynaptically recruiting intrareticular synapses and the potential for network operations in the thalamus to spread corticothalamically induced inhibition at long range through the RTN.

The corticothalamic projection is a powerful stimulus to the initiation of spindle oscillations that are typical of the thalamocortical network during slow wave sleep and drowsy inattentiveness, when RTN cells and relay cells are relatively hyperpolarized (Steriade et al. 1997). The effectiveness of the corticothalamic projection in promoting spindle oscillations appears to depend on its capacity to generate higher amplitude EPSCs in RTN cells than in relay cells, an effect that is likely to depend, in turn, on the enrichment of GluR4 receptor subunits at corticothalamic synapses on RTN cells (Golshani et al. 2001). The more powerful activation of RTN cells ensures that the monosynaptic EPSP in relay cells is quickly overcome by the disynaptic RTN-based IPSP. As relay cells recover from the monosynaptic EPSP in relay cells is quickly overcome by the disynaptic RTN-based IPSP. As relay cells recover from the disynaptic RTN-based IPSP, the resulting hyperpolarization, the low-threshold calcium conductance is released and the relay cells fire a burst of action potentials that reexcite the RTN cells, so promoting continuation of the oscillation. The spread of inhibition through the RTN as more and more RTN cells are excited by the collaterals of thalamocortical fibers may be responsible for the shortening of the burst of discharges of RTN cells that ultimately leads to the fading of spindles as RTN cells and relay cells get out of synchrony (Bal and McCormick 1993; Kim and McCormick 1998). Synchronization of the cells is promoted by the blockade or absence of GABA<sub>A</sub> receptor–mediated inhibition in the RTN, leading to prolonged bursts and hypersynchronized, low-frequency oscillations at 3–4 Hz, resembling absence seizures (Blumenfeld and McCormick 2000; Destexhe 1998; Huguenard and Prince 1994; Sohal et al. 2000; von Krosigk et al. 1993). Shortening of RTN bursts or shunting of excitatory inputs by intrareticular inhibition could be a powerful influence in preventing seizures and corticothalamic activity could reinforce this effect. On the other hand, as pointed out by Sohal et al. (2000), intrareticular inhibition, perhaps enhanced by corticothalamic influences, could also permit focal activity in the thalamocortical network, serving to promote particular spatiotemporal patterns.

Acknowledgments
We thank Dr. S. Hayes and P. Nguyen for technical help.

Grants
This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-59084 and by the W. M. Keck Program in Cellular and Molecular Imaging.

References

*J Neurophysiol* • VOL 91 • FEBRUARY 2004 • www.jn.org


